The Neurobiology of Reward Processing in Adolescence

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'Who in the rainbow can draw the line where the violet tint ends
and the orange tint begins?
Distinctly we see the difference of the colours,
but where does one first blendingly enter into the other?
So with sanity and insanity.'

Herman Melville, Billy Budd Sailor

ABSTRACT

Adolescence represents a time in development when the reward system undergoes substantial changes. Several studies suggest differences in reward processing amongst adolescents compared to adults and children. Abnormalities in reward processing also underlie many psychiatric disorders, such as attention deficit hyperactivity disorder (ADHD). The present research has the following objectives: 1) to investigate normal reward processing during reward anticipation and reward feedback in a large population based cohort of old adolescents. 2) to explore gender differences in reward processing and determine whether the association between reward processing and ADHD symptoms differs between boys and girls. 3) to determine whether the X-linked gene Monoamine Oxidase A (*MAOA)* is associated with ventral striatal brain activation during reward anticipation and 4) to investigate whether *MAOA* stratifies the relationship between ventral striatal activation and ADHD symptoms in boys. Objectives 1 and 2 were explored using the full IMAGEN dataset (n > 1200 adolescent), objective 3 was addressed using the first wave of IMAGEN, including both boys and girls (n = 411 adolescents) whereas objective 4 was investigated using only boys from the first wave (n = 190 adolescents).

The results from random effects analyses and region of interest analyses suggested robust activation patterns during reward anticipation and feedback, particularly in the ventral striatum (VS) and orbitofrontal cortex. Gender differences were prominent during both phases of reward processing with boys showing significantly higher activation of a number of regions, including the VS, relative to girls. We also found that the X-linked gene *MAOA* significantly affected VS activation in boys, but not in girls. This gene also stratified the frequently reported relationship between VS activation and ADHD symptoms in adolescent boys.

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LIST OF ACRONYMS

AAL	Anatomical Automatic Labeling
ADHD	Attention Deficit Hyperactivity Disorder
BOLD	Blood Oxygenation Level Dependent
COMT	Catechol-O-Methyl Transferase (gene)
COMT	Catechol-O-Methyl Transferase (enzyme)
DAT1	Dopamine Transporter 1 (gene)
DNA	Deoxyribonucleic Acid
DRD1-5	Dopamine Receptor D1 (gene)
DSM-IV	Diagnostic Statistical Manual for Mental Disorder, 4th ed.
FEW	Family Wise Error
HWE	Hardy Weinberg Equilibrium
IFG	Inferior Frontal Gyrus
MAOA	Monoamine Oxidase A (gene)
MAOA	Monoamine Oxidase A (enzyme)
MID	Monetary Incentive Delay
MNI	Montreal Neurological Institute
MRI	Magnetic Resonance Imaging
NOS1	Nitric Oxide 1 (gene)
OFC	Orbitofrontal Cortex
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PIQ	Performance Intelligence Quotient
RDH	Reward Deficiency Hypothesis
RDS	Reward Deficiency Syndrome
RNA	Ribonucleic Acid
ROI	Region of Interest
RT	Reaction Time
SDQ	Strengths and Difficulties Questionnaire
SNP	Single Nucleotide Polymorphism
SPM-8	Statistical Parametric Modelling, 8th ed.
SSRT	Stop Signal Reaction Time
SST	Stop Signal Task
TCI	Temperament and Character Inventory
VIQ	Verbal Intelligence Quotient
WISC	Wechsler Intelligence Scale for Children
VNTR	Variable Number Tandem Repeat
VS	Ventral Striatum
VTA	Ventral Tegmental Area

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My PhD is dedicated to my parents who have worked tirelessly to provide my sister and I with all the opportunities we could have wished for in life and have pushed us to be the best we can be. Thank you for always being there for us; without you none of this would have been possible.

DECLARATION

This thesis is based on data from the IMAGEN project which received funding from the European Commission's Sixth Framework Programme (LSHM-CT-2007-037286). My PhD was funded by the Biomedical Research Centre (2009-2012).

Data collection was conducted by Professor Gunter Schumann and the IMAGEN team. Being part of the IMAGEN team, I was responsible for the neuroimaging assessment of ~100 participants at the Institute of Psychiatry, King's College London. I contributed to the visual quality control measures of neuroimaging data and to the behavioural assessment of participants. The expression analysis presented in Chapter Five and Chapter Six was performed by Dr Steven Lubbe. The haplotype analysis presented in Chapter Six was performed by Dr Tianye Jia. The genotyping presented in Chapter Five and Chapter Six was performed by the Centre National de Génotypage in Paris. All data collected for the IMAGEN project is available in a database to which collaborators have access.

I was responsible for generating all hypotheses and research questions in this thesis, conducting statistical analyses and writing up the work for publications. The work presented in this thesis is original and my own. This thesis has not been submitted for any other degree at any other university.

1 CHAPTER ONE:

LITERATURE REVIEW

1.1 INTRODUCTION

Adolescence refers to the developmental time period between childhood and adulthood, considered to take place between the ages of 12 and 17 (Arnett, 1992; Galvan, 2010). This developmental period is distinguished '*by a transition from the dependent, family-oriented state of childhood to the independent, peer-oriented state of adulthood'* (Hardin & Ernst, 2009). This transitional period is frequently marked by increases in a number of characteristic behaviours, including novelty seeking and impulsivity (Arnett, 1992; Steinberg et al., 2008). Whereas some authors have suggested that these behaviours may serve an adaptive function in promoting exploration of the environment and developing skills necessary for independence, they may also result in increased vulnerability to maladaptive behaviours and have been related to several externalising disorders with onset in teenage years.

In support of these statements, research suggest that adolescents have the highest rate of use of virtually every kind of illegal drug (Arnett, 1992). Physical aggression is another troubling behaviour with the proportion of adolescents that engage in minor criminal activity such as minor theft and vandalism, ranging from one-quarter to over three-quarters (Farrington, 1989; Levine & Kozak, 1979). The problematic nature of these behaviours is reflected in studies suggesting that mortality rates amongst adolescents increase by as much as 200% from middle childhood (Dahl, 2004; Spear, 2000). The behavioural changes seen in adolescents are also accompanied by increased onset of psychiatric disorders (Fairchild, 2011). In order to understand the origins of these behaviours researchers have aimed to identify neurobiological mechanisms specific to adolescence.

1.2 IMPULSIVITY AND NOVELTY SEEKING IN ADOLESCENCE

Impulsivity is a risk factor for many common disorders such as ADHD, addictions and antisocial behaviours. Novelty seeking is closely related to impulsivity, but although both may affect risk taking behaviour, they have distinct components (Steinberg, et al., 2008). Impulsivity refers to the lack of self-control or deficiencies in response inhibition, leading to hasty and unplanned behaviour whereas novelty seeking refers to the tendency to seek out novel, varied and highly stimulating experiences and a willingness to take risks in order to attain them (Steinberg, et al., 2008; Marvin Zuckerman, 1979). We still know little about the mechanisms which mediate novelty seeking and impulsivity in adolescents. Studies suggest that immaturities in the brain circuitry mediating reward processing may predispose adolescents to novelty seeking and impulsive behaviours (C. Geier & Luna, 2009; C. F. Geier, Terwilliger, Teslovich, Velanova, & Luna, 2010; Telzer, Fuligni, Lieberman, & Galvan). It has been suggested that an immature reward system may lead adolescents to wrongly assess the risks which accompany an action or stimulus. For example, an adolescent whose reward system is not fully developed may decide to engage in a risky action, such as taking drugs, stealing or driving drunk, in order to activate an otherwise sluggish reward system (Bjork et al., 2004). However, others suggest that the adolescent reward system is overactive and results in increased impulsivity and novelty seeking (Galvan, Hare, Voss, Glover, & Casey, 2007; C. Geier & Luna, 2009). The hypothesis that the adolescent reward system is overactive is often combined with a second component of reward seeking behaviour in adolescents, namely the idea that adolescents suffer a lack of inhibitory control (Ernst & Fudge, 2009; C. F. Geier, et al., 2010). This idea stems from evidence suggesting that the prefrontal cortex has not yet matured to the point where risks can be

sufficiently assessed. In particular, the connections between the prefrontal cortex and other cortical regions have not fully developed in adolescence (Blakemore & Choudhury, 2006). This may result in inadequate control over reward-related impulses.

The development of the reward and inhibitory system are beginning to be investigated in humans. Here, I will review literature on normal functioning as well as studies of the maturation of reward processing and inhibitory control.

1.3 MEASURING REWARD PROCESSING IN NON-HUMAN PRIMATES

Behavioural studies of animals suggest that a stimulus that lacks intrinsic rewarding value, for example the sound of a bell, can become rewarding in its own right if it is repeatedly paired with a rewarding stimulus, such as food (i.e. an unconditioned stimulus). Once the stimuli have been successfully paired, the stimulus without intrinsic reward value becomes rewarding in its own right (and has become a so-called conditioned stimulus). Thus, the sound of the bell becomes a cue which triggers anticipation of the delivery of a reward.

Based on behavioural conditioning experiments of animals, a neurological connection between anticipation and receipt of reward was hypothesised. This connection was determined through single cell studies of non-human primates. Studies of the macaque monkey suggest that dopamine neurons in the ventral tegmental area (VTA) would respond with short, phasic activations when the monkey is presented with various appetitive stimuli, such as fruit juice (Schultz, Dayan, & Montague, 1997). When this rewarding stimulus was preceded by a visual or auditory cue the dopamine neurons would change the time of activation from just after the time of reward delivery to the time of cue onset. Based on this information it was

concluded that the dopamine neurons had learned the association between the cue and the reward and responded to the earliest possible event prior to the reward, i.e. the cue.

Based on this information neuroscientists suggested that reward processing could be divided into two temporally distinct phases, one prior to reward delivery (i.e. the reward anticipation phase) and one after reward delivery (Schultz, et al., 1997). The signals measured prior to reward delivery, in response to a cue, are thought to reflect reward detection as well as estimation of the valence and anticipated value of the future reward. Signals occurring after reward delivery are thought to relate to the magnitude and valence of the received reward.

1.4 Neuroimaging of Reward Processing in Humans

Temporal aspects of reward processing fMRI studies frequently use the monetary incentive delay (MID) task, which was developed by Brian Knutson and colleagues, to detect BOLD responses associated with reward-related neural mechanisms (Knutson, Adams, Kaiser, Walker, & Hommer, 2000).

The MID task is an event-related task designed to measure brain activation while a person anticipates making a simple motor response in order to gain a reward, but it also measures activations during the receipt of a reward. During the MID task the participant first sees a cue that indicates what is at stake in the current trial, e.g. a small or large amount of money or points. Next, the subject presses a button when he or she sees a target appear briefly. In most versions of the task the button must be pressed while the target is present. The duration of the target's appearance varies so that the subject is successful in only a set number of trials. Finally, the participant receives feedback indicating whether he or she successfully responded to the target and how much was won on the particular trial. Thus, the MID task measures brain

activation during two phases of reward processing: the reward anticipation phase and the outcome phase, also known as reward feedback. Below I will discuss what is known about immature reward processing, but first I will provide an overview of the adult reward system.

1.5 THE ADULT REWARD SYSTEM

Reward processing in adults has been fairly well investigated using the MID task (Knutson, et al., 2000; Knutson, Fong, Adams, Varner, & Hommer, 2001; Knutson, Taylor, Kaufman, Peterson, & Glover, 2005; Liu, Hairston, Schrier, & Fan, 2011). The MID task reliably activates subcortical regions of the basal ganglia. The basal ganglia consist of several structures including the caudate, putamen, nucleus accumbens, the globus pallidus, the subthalamic nucleus and the substantia nigra. The striatum receives afferents from different limbic regions, including the ventral tegmental area, which is the main projector of dopamine (Delgado, 2007; Knutson, Adams, Fong, & Hommer, 2001; Schultz, et al., 1997).

The striatum can be further subdivided into a dorsal and a ventral component. The dorsal component consists of the caudate nucleus and putamen, which connect to motor and prefrontal regions (Delgado, 2007). The ventral striatum (VS), which consists of the nucleus accumbens as well as ventral portions of the caudate nucleus and putamen, is connected to ventral regions of the prefrontal cortex, through the mesolimbic pathway, which is thought to be involved in emotion and motivation. Dopamine is an important neurotransmitter in the brain's reward system, and particularly in the striatum (Delgado, 2007; Schott et al., 2008; Schultz, et al., 1997). A key assumption underpinning many functional MRI studies of reward processing is that ventral striatal activation reflects dopaminergic signalling. This assumption was proven in a study by Schott and colleagues that measured synaptic dopamine levels

during the MID task, using positron emission tomography (PET), in combination with activation patterns measured by functional MRI. The study showed a positive correlation between synaptic dopamine levels measured by PET and VS activation measured by fMRI during reward anticipation, suggesting that activation of the VS reflects dopaminergic transmission (Schott, et al., 2008).

The striatum is connected with the orbitofrontal cortex (OFC) through the mesocortical and mesolimbic pathways (Delgado, 2007; Liu, et al., 2011). The OFC has been suggested to mediate reward- and punishment-guided aspects of behaviour, but more recently it was suggested that the medial OFC is important in making value-guided decisions and in assigning credit for rewards (Noonan, Kolling, Walton, & Rushworth, 2012). Several studies suggest the striatum is mainly activated during reward anticipation, whereas the OFC makes value-based decisions based on feedback information during the outcome/feedback phase of reward processing (Kringelbach, 2005; Noonan, et al., 2012; Sescousse, Redoute, & Dreher, 2010).

Whereas the striatum and OFC are most frequently associated with reward processing, several other regions have also been implicated. Studies suggest that the cingulate cortex is activated during both phases of reward processing (Knutson & Cooper, 2005; Knutson, Fong, et al., 2001). Evidence suggests that activation of the anterior cingulate cortex during reward anticipation encodes the potential value of an action. During reward feedback, anterior cingulate activity encodes the degree to which information about the reward should influence future actions and decisions (M. Rushworth, Behrens, & Walton, 2008; M. F. S. Rushworth & Behrens, 2008). Similar to the role of the anterior cingulate, the parietal lobule has been associated with the valuation of options and information integration. Studies suggest that the parietal lobule show greater activation during reward anticipation relative to the feedback

phase of reward processing. It is believed that this region is involved in planning and preparing informed actions during reward anticipation (Liu, et al., 2011). Finally, activation of the precentral gyrus is frequently identified during reward anticipation. However, few studies attempt to explain the role of this region during reward processing. It is suggested that the precentral gyrus plays an important role in preparation for action during reward anticipation (Ernst et al., 2004), but more recent studies suggest that cognitive and motivational signals interact in this region (Padmala & Pessoa, 2010).

1.6 MODELS OF ADOLESCENT REWARD PROCESSING

Whereas the adult reward system is fairly well understood, fewer studies have specifically focused on the maturation of the human reward system (C. Geier & Luna, 2009; C. F. Geier, et al., 2010). It is suggested that there are differences in how adolescents and adults process rewards and that these differences are associated with risk taking behaviour. Two models have emerged from the literature; both suggest that adolescents use the same underlying brain circuitry to process rewards as adults do. However, the models differ with regards to whether this circuitry is under- (hypo) or over- (hyper) activated during reward processing.

Both models focus particularly on activation in the VS, which is a key reward-region that receives dopaminergic afferents from the ventral tegmental area. The first model suggests that the VS is hypoactive and thus less strongly recruited during reward processing in adolescents than it is in adults (Bjork, et al., 2004; C. Geier & Luna, 2009). According to this model, risk taking is the result of adolescents seeking out risky activities and situations in order to boost the activation in an otherwise sluggish reward system. For the same reason adolescents may be more prone to engage in substance use in order to compensate for the low activation of the reward

system. This model is also connected to the Reward Deficiency Hypothesis (Blum, Cull, et al., 1996), which suggests that risk taking behaviour is the result of reduced activation of the reward system (see **Box 1**).

The opposing model suggests that the reward system of adolescents is hyperactive, meaning that the VS shows increased responsiveness to rewards compared to adults (Ernst et al., 2005; Galvan, et al., 2007; Galvan et al., 2006). Studies of dopaminergic function in adolescents also suggest an increase in cortical dopaminergic release during adolescence (Chambers, Taylor, & Potenza, 2003). It is suggested that this increase in VS activation serves an adaptive function as it would increase novelty seeking behaviours that may promote the independence necessary in adulthood. This model is also related to the triadic model, which suggests that adolescent risk taking is the result of interactions between a hyperactive VS combined with limited amygdala activation (mediating harm-avoidance) and prefrontal activation (mediating inhibitory control) (Ernst, et al., 2005).

Studies that have investigated the development of reward processing suggest that adolescents engage similar neural circuitry as adults, including the dorsal and ventral striatum, OFC and amygdala. The divergence between the most commonly cited studies of reward processing in adolescence are presented in ***Table 1***. May and colleagues performed the first event-related functional MRI study to determine whether adolescents and children activate comparable regions as adults during reward processing (May et al., 2004). The study design investigated brain responses to monetary gains and losses in 18 healthy adolescents and children between the ages of 8 and 18 years. This study showed that children and adolescents recruit the VS and OFC during the anticipation and loss of rewards. However, the study did not include

an adult comparison group. Therefore, it was unable to conclude whether adolescents and adults show significant differences in reward processing.

Bjork and colleagues performed a study comparing brain responses of adults and adolescents while gaining and losing reward during the MID task (Bjork, et al., 2004). The results suggested that despite similar behavioural performances on the MID task, adolescents, aged 12-17 showed less VS activation in anticipation of reward compared to adults, aged 22-28. However, no group differences were found during reward feedback. The authors conclude that the increase in risky behaviours frequently seen amongst adolescents may be 'a way of compensating for low ventral striatal activity'.

Whereas Bjork and colleagues failed to identify age differences during reward feedback this stage of reward processing was targeted in a study by Ernst and colleagues (Ernst, et al., 2005). Their study of 14 adults (20-40 years) and 16 adolescents (9-17 years) responses to reward receipt and omission suggested that adolescents show higher activation of the VS and amygdala during reward receipt and reward omission compared to adults. Thus, the study supports the triadic model, which suggests that adolescent risk taking is the result of an imbalance between the reward-oriented ventral striatal activation and harm-avoidant amygdala activation.

A study by Galvan and colleagues investigated differences in reward processing between 16 children, 13 adolescents and 12 adults (Galvan, et al., 2006). In contrast to findings by Bjork and colleagues, this study suggested that adolescents showed significantly higher activation of the VS during reward anticipation compared to both adults and children. In a follow-up study of children, adolescents and adults aged 7-29 years, Galvan and colleagues also suggested that higher VS activation across ages was associated with increased impulsivity, suggesting that increased

reactivity of the VS during reward anticipation is correlated with higher impulsivity scores (Galvan, et al., 2007). Several reasons have been suggested to explain the discrepancies between studies suggesting that the reward system of adolescents is hyper- vs. hypo-activated (Galvan, 2010). These are presented below.

1.6.1 Defining Adolescence

Adolescence can be defined by age, pubertal development or educational grade. Thus, adolescence can be hard to define in scientific terms and studies differ in terms of who they include as adolescent participants. Several studies mentioned above (Bjork, et al., 2004; Ernst, et al., 2005; May, et al., 2004) included 12-year olds in their studies. Whereas a 12-year old may be considered a young adolescent, a 12-year old is probably at a very different stage of development compared to a 17-year old. Targeting adolescents within a homogeneous age range may further our understanding of the development of the reward system.

1.6.2 Task Analysis

The difference between the results found by the studies may be due to which part of the MID task is analysed. The BOLD-responses that occur before and after reward delivery are distinct. Whereas anticipatory signals are associated with the initial detection and determination of the valence of reward cues, signals of reward feedback are associated with whether the received reward matched up with predictions. Whereas the study by Ernst and colleagues found that the VS was hyperactive in adolescents compared to adults during reward feedback, the studies by Bjork and colleagues and Galvan and colleagues targeted developmental differences in VS activation during reward anticipation (Bjork, et al., 2004; Ernst, et al., 2005; Galvan, et al., 2006). However, the studies that target reward anticipation differ in whether the

adolescents showed higher activation compared to adults or lower activation compared to adults.

1.6.3 TASK DESIGN

The studies mentioned above use a wide range of tasks to engage the reward system. In particular, the developmental appropriateness of these tasks has been discussed. The studies all investigate differences in BOLD-responses between adults and adolescents.

Whereas the MID task is a very simple task, it is also assumed that adolescents will find this task as engaging as adults do. Bjork and colleagues investigated developmental differences in reward processing using the standard MID task targeting VS responsivity to both gains and losses of rewards (Bjork, et al., 2004). The study showed no differences in performance or reaction time data between adults and adolescents, but the study has been criticised for using the standard MID task, which was initially developed for adults. Galvan and colleagues designed an age-appropriate task to measure reward processing (Galvan, et al., 2006). This task used cartoon-like stimuli and described the task as a videogame. While the study by Galvan and colleagues found that the VS of adolescents was hyperactivated, the study by Bjork and colleagues suggested that the VS of adolescents was hypoactivated during reward anticipation (Bjork, et al., 2004; Galvan, et al., 2006). Task design may explain the differences observed between these studies.

Table 1. Functional MRI studies of reward processing in adolescence supporting either the Reward Deficiency Hypothesis or the Impulsivity Hypothesis

Authors	***Main Findings***	***Adolescent group: Gender and Age***	***Comparison Group: Gender and Age***	***Task Design***	***Analysis Focus***	***Supporting RDS or Impulsivity Hypothesis***
Bjork et al. 2004	Adolescents show reduced activation of VS relative to adults	Adolescents: N = 12 (6 males), 12-17 years	Adults: N=12 (6 males), 21-28 years	Reward magnitude	Anticipation of reward	Supporting RDS
May et al. 2004	Adolescents and children activate the VS and OFC during reward processing similarly to adults	Adolescents: N = 12 (5 males), 8-18 years	No comparison group (results were compared to prior literature)	Reward probability	Entire trial	N/A
Ernst et al. 2005	Adolescents show increased activation of the VS relative to adults	Adolescents: N = 16, (gender of participants were not stated) 9-17 years	Adults: N = 14 (gender of participants were not stated), 20-40 years	Reward magnitude	Feedback of reward	Supporting Impulsivity Hypothesis
Galvan et al. 2006	Adolescents show increased activation of the VS relative to children and adults	Adolescents: N = 13 (7 males) 13-17 years	Children: N = 16 (9 males), 7-11 years and Adults: N = 12 (6 males), 23-29 years	Reward magnitude	Anticipation of reward	Supporting Impulsivity Hypothesis

1.7 RESPONSE INHIBITION

Immature reward processing is unlikely to be the only determinant of adolescent behaviour. In parallel with changes in reward processing, inhibitory control mechanisms also undergo maturation during adolescence. The hyperactivity model of adolescent reward processing is frequently discussed in combination with the triadic model proposed by (Ernst, et al., 2006). The triadic model suggests that impulsive and novelty seeking behaviours is the result of an imbalance between three neural systems: i) the reward system mediated by VS activation; ii) the harm avoidance system mediated by amygdala activation and iii) the regulatory system mediated by prefrontal, and particularly inferior frontal, activation. The triadic model suggests that adolescents show increased activation of the VS during reward processing and deficient prefrontal activation during response inhibition (Ernst, et al., 2006). Failure to inhibit responses may result in inappropriate reward seeking behaviour due to poor control of impulses from the reward system (see **Box 2**). Understanding the development of normative response inhibition may provide insight on basic mechanisms contributing to the emergence of risk taking. Below, I will present an overview of how response inhibition is targeted through neuroimaging methods, as well as some key findings regarding the functioning of the mature and maturing inhibitory system.

1.8 NEUROIMAGING OF RESPONSE INHIBITION

Inhibitory control is an important component of executive function that allows humans and animals to suppress the processing of information that would disrupt efficient completion of a task at hand. Response inhibition has been defined as 'the ability to deliberately suppress defined automatic, or prepotent responses'(Friedman & Miyake, 2004). Inhibitory control plays an important role in memory, attention and

intelligence. The impairment of inhibitory control is a core feature of many psychiatric disorders, such as Attention Deficit Hyperactivity Disorder, Obsessive Compulsive Disorder and Schizophrenia. Through simple tasks, we can measure how the brain responds when it succeeds or fails to suppress an unwanted response (Aichert et al., 2012).

The inhibitory system is engaged when deciding among competitive alternatives during decision making and is believed to play an important role in reward-based decision making. Tasks that examine response inhibition involve routine responses to a frequently shown cue, such as an arrow pointing left or right. These routine responses may be followed by an infrequent stop cue, such as an arrow pointing upwards. The participant then has to abort the routine response by making an effortful mental cancellation of the planned action (Hampshire, Chamberlain, Monti, Duncan, & Owen, 2010).

Several types of tasks have been developed to study different aspects of response inhibition. The withholding of a routine response is frequently studied using the Go/No Go task, the suppression of a response that may have already started is typically investigated using the stop signal task (SST), and the protection from cognitive interference is examined using different versions of the Stroop task (Aron, Robbins, & Poldrack, 2004; Hampshire, et al., 2010; Rubia, Smith, Brammer, & Taylor, 2003).

The SST, used in the studies presented in this thesis, is composed of Go trials and Stop trials. During Go trials the participants are presented with arrows pointed right and left for which they have been instructed to give simple motor responses, by pressing a button. In the unpredictable and infrequent Stop trials, the arrows pointing left or right are followed by arrows pointing upwards. During these trials, the

participant has to inhibit the routine motor response. In order to inhibit responses during Stop trials a number of brain regions are activated.

1.9 THE ADULT INHIBITORY CONTROL SYSTEM

A distributed neural network is believed to underlie response inhibition, including the inferior frontal gyrus, the cortical eye field, anterior cingulate cortex and basal ganglia as shown in functional imaging work in humans. The basal ganglia plays an important role in Go trials as it mediates approach. Go trials have been associated with activation of the basal ganglia-thalamocortical circuit, which is thought to play a role in movement control and adaptive motor behaviour (Alexander, Crutcher, & Delong, 1990). Results from the SST and Go/No Go tasks suggest that the right inferior frontal gyrus plays the most prominent role in inhibiting routine responses as measured during Stop trials. Functional MRI data reveals increased right IFG activation during Stop trials relative to a baseline of routine responses (Hampshire, et al., 2010; Rubia, et al., 2003). For the SST, the index of inhibitory control is the duration of the stopping process, called the stop signal reaction time (SSRT). Damage to the right IFG affects performance on the SST by disrupting inhibition. The greater the damage to the right IFG, the worse the response inhibition as indexed by the SSRT. Several studies suggest correlations between right IFG BOLD-response and stop signal reaction times (Aron, et al., 2004; C. S. R. Li, Huang, Constable, & Sinha, 2006; Swick, Ashley, & Turken, 2011).

1.10 THE ADOLESCENT INHIBITORY CONTROL SYSTEM

Aspects of cognitive control and response inhibition in particular, are believed to develop in parallel with reward processing. The maturation of response inhibition may play a significant role in how rewards guide behaviour and decision-making. For

example, an immature inhibitory system may bias adolescents to respond to an immediate reward, even if that means neglecting a larger reward that is delivered later.

Several studies suggest that inhibitory control of behaviour continues to improve well into adolescence. Adolescents show improved performance during SST, stroop tasks and Go/No Go tasks compared to children (Levin et al., 1991; Liston et al., 2006; Williams, Ponesse, Schachar, Logan, & Tannock, 1999). Functional MRI studies also suggest that adolescents activate the bilateral inferior frontal cortex to No Go stimuli, but adults have greater and more focal activity, particularly in the right hemisphere (Stevens, Kiehl, Pearlson, & Calhoun, 2007; Tamm, Menon, & Reiss, 2002).

In cases where children and adolescents perform at adult levels on the SST it is suggested that the greater activity of the inferior frontal cortex may reflect the need to overcome relatively weak anatomical connections among key brain regions through greater top-down executive control (Stevens, et al., 2007). This is consistent with the hypothesis that improvements in performance across development may result from ongoing prefrontal specialisation and connectivity among prefrontal and subcortical regions with increasing age (Rubia, Smith, Taylor, & Brammer, 2007). This hypothesis was verified in a study by Stevens and colleagues who showed that adolescents differed from adults in the degree of fronto-striatal-thalamic connectivity, which may in turn affect response inhibition in adolescents (Stevens, et al., 2007).

1.11 GENDER DIFFERENCES

1.11.1 GENDER DIFFERENCES IN PSYCHIATRIC DISORDERS

Several studies suggest that adolescent boys are substantially more novelty seeking and impulsive than girls. A study by Romer and Hennessy suggested that novelty

seeking increased rapidly from the age of 14 until it peaked at age 16 years in girls and 18.5 years in boys (Romer & Hennessy, 2007). The earlier peak in girls is consistent with the tendency for puberty to emerge earlier in girls and observed effects on brain maturation.

Considering that novelty seeking and impulsivity are thought to be related to reward seeking it is interesting that personality questionnaire data suggest gender differences in sensitivity to reward and reward dependence. In the Cloninger's United States normative data, women scored higher than men on reward dependence (Cloninger, Przybeck, & Svrakic, 1991). These findings supported previous work by (Nixon & Parsons, 1989). Other personality questionnaire studies, using the sensitivity to punishment and sensitivity to reward questionnaire (SPSRQ), suggest that men score significantly higher on the scale of reward sensitivity relative to females.

Gender differences are also studied in reward sensitivity and reward-related disorders, which frequently have their onset in adolescence. It is believed that some of the biological and reproductive processes, which make males and females different, also make individuals different in the way they respond to everyday rewarding stimuli. Males often show higher levels of externalising problems, which are frequently linked to the reward system. These problems include addictions, ADHD and antisocial behaviours. Gender differences become particularly pronounced during adolescence, when boys tend to display more antisocial behaviour and conduct problems (Hicks et al., 2007). Females show a significantly higher level of internalising disorders such as depression, but also of eating disorders (Ormel et al., 2005). In order to understand gender differences in disorders of reward

sensitivity, neuroscientists have begun to examine gender differences in the brain circuitry underlying reward processing.

1.11.2 Gender Differences in Reward Processing

Whereas several studies have identified gender differences in brain size and cranial tissue compartments, few studies investigate gender differences in BOLD-responses measured through functional MRI. One recent study used functional MRI to investigate gender differences in a modified version of the MID task where the participants could expect to win either money or positive social feedback, in the form of smiling faces. This task was called the social incentive delay (SID) task. Whereas males showed the usual activation of mesolimbic brain regions during anticipation of monetary rewards, they showed very little activation during anticipation of social rewards. In contrast, females showed identical activation of reward regions during anticipation of monetary rewards and social rewards. In the SID task women showed stronger activation in response to increasing levels of anticipated rewards than men in the right caudate. The opposite comparison (MID: women > men and SID: women > men) did not reveal any effects (Spreckelmeyer et al., 2009). While this finding is interesting in terms of understanding individual differences in reward valuation, the study investigated reward processing in a small sample of 16 male and 16 female adults, rather than adolescents.

Gender differences in the reward system have also been shown in a positron emission tomography (PET) study, suggesting that men show a significantly higher level of striatal dopamine release compared to females after taking amphetamine. These findings may explain why males are more likely to engage in addictive behaviours than females (Munro et al., 2006). Similar results have been found in mice, suggesting that gender differences in striatal dopamine release is the result of

the gonadal hormones estrogen and progesterone modulating dopamine concentration of striatal estrogen in amphetamine stimulated dopamine release (Becker, 1999).

Pubertal hormones have been shown to affect the way we respond to rewards. In females, reward-sensitivity is associated with the menstrual cycle. Healthy female volunteers show greater frontostriatal responses to monetary rewards in the follicular phase of the cycle, which is the phase when estrogen is unopposed by progesterone compared to the luteal phase when progesterone levels are high (Caldu & Dreher, 2007). Testosterone has also been shown to influence performance on the Iowa Gambling Task thought to indicate reduced reward sensitivity (van Honk et al., 2004).

1.12 Neural Mechanisms of Attention Deficit Hyperactivity Disorder

Attention Deficit Hyperactivity Disorder (ADHD) is a disorder of impulsivity, hyperactivity and inattention. Recent studies suggest that ADHD may be better conceptualized dimensionally than categorically. However, the approved 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) suggests that ADHD will continue to be diagnosed categorically Most recently Marcus and Berry (2012) investigated the latent structure of ADHD in order to determine whether the disorder is most validly treated through categorical or dimensional models. The study showed that inattention, hyperactivity/impulsivity and ADHD symptoms all have a dimensional latent structure. Overall, treating ADHD continuously accounted for 2.6 times as much variance as treating ADHD categorically.

Deficits in reward processing and inhibitory control are frequently suggested to underlie the behavioural characteristics of ADHD. In order to better understand the neurocognitive mechanisms which underlie the disorder, several studies have used

functional MRI. Functional MRI studies suggest that deficits in reward processing and inhibitory control jointly or independently contribute to the disorder. The dual pathway model of ADHD suggests that for some ADHD patients the origins of the disorder may lie in a deficit of the reward system whereas for others the disorder stems from a deficit in inhibitory control (E. Sonuga-Barke, Bitsakou, & Thompson, 2010; E. J. S. Sonuga-Barke, 2002). The model suggests that ADHD is caused by 'at least two relatively independent, but mutually exclusive ADHD endophenotypes' (Carmona et al., 2011), suggesting that a person could suffer from ADHD due to disruptions in inhibitory control or abnormalities in the reward system or by an interaction of the two. Here, I will present studies that have investigated the separate effects of deficient reward processing and response inhibition on ADHD. Finally, I will present a study by Carmona and colleagues, which investigated reward processing and response inhibition in an intrasubject design of ADHD patients and healthy controls. A summary of the studies presented below can be found in ***Table 2***.

1.12.1 ENDOPHENOTYPES

Endophenotypes have been defined as measurable components unseen by the unaided eye along the pathway between disease and distal genotype, which may be neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, or neuropsychological (including configured self-report data) in nature. They are thought to represent simpler clues to genetic underpinnings than the disease syndrome itself.

Intermediate phenotypes, such as neuroimaging measures, are believed to be biologically 'closer' to the genotypes and more heritable than the classical diagnoses of disorders (Gottesman & Gould, 2003). Due to their continuous nature,

neuroimaging measures are arguably more sensitive and biologically robust measures than categorical diagnoses. However, to ascertain their usefulness as endophenotypes, assessment of heritability of structural and functional neuroimaging phenotypes is critical.

Whereas studies show heritability estimates of volumetric measures of brain tissue, suggesting that genetic factors account for 70-90% of the variance in total cerebral volume and grey and white matter volumes less research has identified heritability estimates of phenotypes derived from functional MRI (Giedd, Schmitt, & Neale, 2007). Larger twin model neuroimaging studies are needed to determine the heritability to brain function in areas such as reward processing. Such studies may explain which aspects of brain function and structure are heritable.

1.12.2 Deficits in Reward Processing

Growing evidence suggests that the reward system of ADHD patients is dysfunctional in comparison to healthy controls. The behaviour of ADHD patients of all ages is frequently described to be driven by immediate reward with reduced long-term value over delayed rewards with higher long-term value (Solanto et al., 2001). Furthermore, studies suggest that children with ADHD require stronger incentives in order to modify behaviour and learn faster by direct reinforcement (Kollins, Shapiro, & Abramowitz, 1998; Strohle et al., 2008).

Studies have investigated functional abnormalities in reward processing in adolescents and adults with ADHD. Scheres and colleagues investigated VS activation using the MID task in 11 adolescents diagnosed with ADHD compared to 11 healthy controls, aged 12-17 years (Scheres, Milham, Knutson, & Castellanos, 2007a). The study found that adolescents with ADHD showed reduced VS activation during the reward anticipation phase of the MID task. VS activation was also

negatively correlated with parent-rated hyperactivity/impulsivity scores. Scheres and colleagues had postulated that the VS would be hyperresponsive during the reward feedback phase. However, adolescents with ADHD and healthy controls did not differ in their VS activation in response to reward feedback.

A later study by Strohle and colleagues used functional MRI to compare neural responses to reward anticipation and feedback in 10 male adults with ADHD and 10 male healthy controls (Strohle, et al., 2008). This study suggested that adults with ADHD show decreased activation of the VS during the anticipation phase of the MID task, but increased activation of the OFC in response to the feedback phase. Again, VS activation during reward anticipation was negatively correlated with symptoms of hyperactivity and impulsivity.

These studies suggest that ADHD patients, regardless of age, show lower activation of the VS during reward anticipation. Studies by Scheres and Strohle also revealed negative correlations between measures of ADHD symptoms and VS activation, suggesting that VS-activation patterns during reward anticipation is sensitive to both categorical and continuous measures of ADHD symptoms (Scheres, et al., 2007a; Strohle, et al., 2008). Due to deficient responses to rewards, ADHD is sometimes considered a reward deficiency syndrome (see **Box 1**). However, it is also considered a disorder of deficient inhibitory control. It is suggested that deficiencies in reward processing and inhibition jointly result in ADHD (see **Box 2**).

1.12.3 Deficits in Response Inhibition in ADHD

Functional MRI studies have revealed that activation within the right IFG increases at the point of inhibitory control when compared to baseline response. However, studies differ with regard to the whether the association of ADHD symptoms and IFG activation is positive or negative during successful inhibition (i.e. whether the right

IFG is hyper- or hypo-activated during response inhibition). A few studies provide evidence of reduced activation in the inferior frontal gyrus in ADHD during successful response inhibition.

Rubia and colleagues performed a functional MRI study of response inhibition in a sample of 16 male medication-naïve adolescents with ADHD and 21 matched controls (9-16 years) (Rubia, Smith, Brammer, Toone, & Taylor, 2005). The authors used the SST to determine whether the IFG is hyper- or hypo-activated in ADHD patients. Results suggested that the IFG of ADHD patients was hypoactivated during inhibition Stop trials compared to the IFG of healthy controls. Rubia and colleagues also showed that this hypoactivation was specific to ADHD as it did not occur amongst individuals suffering from conduct disorder (Rubia et al., 2008). However, others have found that ADHD patients show a hyperactivation of the inferior frontal gyrus during successful inhibition. Using a Go/No Go task, Schultz and colleagues showed that adolescents with childhood ADHD (n = 10) showed significantly higher activation of the IFG compared to adolescents with no history of ADHD (n = 9) (Schulz et al., 2004). These findings were supported by Pliszka and colleagues who investigated cortical responses to the SST in 17 children with ADHD and 15 healthy control subjects (9-15 years) (Pliszka et al., 2006). The study suggested that the ADHD patients activated the IFG more on Stop trials relative to healthy controls. The discrepancies may result from differences in treatment amongst participants as the study by Rubia and colleagues used only medication naïve participants, whereas participants in the other two studies had a history of long-term treatment with stimulants (Rubia, et al., 2005).

The first, and to-date, only study to investigate reward processing and response inhibition in the same participant group, was recently presented by Carmona

and colleagues (Carmona, et al., 2011). In order to test the dual-pathway model, this study used an intrasubject design to assess whether adults with ADHD exhibited neurological disturbances during response inhibition, reward anticipation or both tasks. The study tested whether disturbances in the reward and inhibitory systems are independent of each other, as suggested by the dual-pathway model. The results suggested that ADHD patients showed significantly reduced VS activation during reward anticipation relative to controls. However, they found no significant differences in right IFG activation during response inhibition in ADHD patients compared to controls. The results confirm the hypothesis that VS reward-related activation and right IFG response inhibition can contribute to ADHD as relatively independent processes. However, further research is needed to determine whether this is the case across development.

Understanding how deficits in the reward and inhibitory control systems contribute to ADHD is important in order to improve treatments for the disorder. However, the brain activation patterns identified during these tasks can also facilitate genetic investigations of ADHD. It is believed that intermediate phenotypes, such as the brain regions activated during reward processing or inhibitory control are simpler outcome measures than the disorder itself. Most importantly, it is believed that a fewer number of genes will play a role in these intermediate phenotypes than in the complete clinical construct. Below, I will give a brief overview of the genetics of ADHD before presenting how imaging genetic studies may improve our understanding of the disorder.

Table 2. Functional MRI studies of reward processing and inhibitory control in ADHD patients, supporting either the Reward Deficiency Hypothesis or the Impulsivity Hypothesis

Authors	***Main Findings***	***Participants***	***Task Design***	***Analysis Focus***	***Supporting RDS or Impulsivity Hypothesis***
Schultz et al. 2004	Adolescents with childhood ADHD showed reduced activation of the IFG in comparison to adolescents with no history of ADHD	Adolescents with childhood ADHD: N = 10 (9 male) Matched healthy controls: N = 9	Go/No Go	Response inhibition	Supporting Impulsivity Hypothesis
Pliszka et al. 2006	ADHD patients showed increased activation of IFG on Stop trials relative to Go trials when compared to healthy controls	Children with ADHD: N = 17 (Gender not stated) Matched healthy controls: N = 15	Stop Signal	Response inhibition	Supporting Impulsivity Hypothesis
Rubia et al. 2005	Medication-naïve adolescents with ADHD show reduced activation of IFG during unsuccessful response inhibition	Male medication-naïve adolescents with ADHD: N = 21 Matched healthy controls: N = 16	Stop Signal	Response inhibition	N/A
Scheres et al. 2007	Adolescents with ADHD show reduced activation of the VS during anticipation of rewards relative to controls	Adolescents with ADHD: N = 11 (Gender not stated) Matched healthy controls: N = 11	MID Task	Anticipation of reward	Supporting RDS

Authors	*Main Findings*	*Participants*	*Task Design*	*Analysis Focus*	*Supporting RDS or Impulsivity Hypothesis*
Strohle et al. 2008	Adults with ADHD show reduced activation of the VS during anticipation of reward and increased activation of OFC during feedback of reward relative to controls	Male adults with ADHD: N = 10 Matched healthy controls: N = 10	MID Task	Anticipation and feedback of reward	Supporting RDS
Carmona et al. 2011	Adults with ADHD show reduced activation of the VS during anticipation of reward relative to controls, but no difference in IFG activation	Male adults with ADHD: N = 23 Matched healthy controls: N = 23	MID Task and Go/No Go Task	Anticipation of reward and response inhibition	Supporting RDS

1.12.4 GENETICS OF ADHD

Genetically sensitive designs indicate that the heritable foundation of ADHD is substantial. Family studies show that a significantly higher rate of the disorder is found in probands of individuals with ADHD (11%) than in the general population (5%) (Leckman, Weissman, Pauls, & Kidd, 1987). Twin studies estimate that genetic components account for 60-80% of the variability within the disorder and non-shared environmental effects account for 20-40% of the variability within the investigated phenotype (Faraone & Doyle, 2001; Faraone et al., 2005; Nikolas & Burt, 2010). However, identifying the specific underlying genetic risk factors contributing to the disorder has proven difficult. As such ADHD conforms to the characterization of most psychiatric disorders: i.e. it is not inherited according to a simple Mendelian, single-gene pattern, but is assumed to be caused by numerous genes of small effect sizes (Plomin, 2008).

To date no genetic polymorphism has been identified as necessary or sufficient to develop ADHD; however, a number of candidate genes have been the focus of study. Since ADHD is a behavioural disorder, genes encoding enzymes involved in brain dysfunction, and particularly genes serving an excitatory function, are obvious candidates for research (Gizer, Ficks, & Waldman, 2009). A full review of the genetics of ADHD is beyond the scope of this introduction; however, some important findings are referred to below.

Genetic variants influencing the reward and inhibitory pathways of the central nervous system are of particular interest, these genes are often involved in transmission, reception and degradation of dopamine are frequently investigated. The dopamine transporter gene (*DAT1;* also known as *SLC6A3*) is the most frequently studied candidate gene in association with ADHD (Gizer, et al., 2009). The 10 and 9

repeats of *DAT1* were initially associated with ADHD in a study of 57 children which suggested that the 10 repeat allele was preferentially transmitted to ADHD probands (Cook et al., 1995). Over 100 studies have now examined the relationship between *DAT1* and ADHD and several SNPs within the gene have also been shown to contribute to ADHD (Gizer, et al., 2009).

Two dopamine receptor genes have also been of particular interest, namely *DRD4* and *DRD2*. A variable number tandem repeat (VNTR) in *DRD4* is most commonly associated with ADHD. Studies suggest that the 7 repeat allele is a functional polymorphism that is frequently demonstrated to be associated with ADHD. Several studies also suggest that this allele is associated with poor performance on neuropsychological measures (Kieling, Roman, Doyle, Hutz, & Rohde, 2006; Langley et al., 2004). The Taq1 polymorphism within *DRD2* is expressed in several brain regions thought to be important for reward processing in ADHD (Blum & Noble, 1990). Several studies suggest that *DRD2* Taq1 is associated with ADHD. However, controversy still exists regarding which allele transmits risk for the disorder (Kirley et al., 2002; Kopeckova et al., 2008). Whereas the evidence for the association between *DRD2/DRD4* and ADHD is convincing, there is less consistency in studies linking *DRD1*, *DRD3* and *DRD5* to ADHD.

The degradation of dopamine and serotonin is also thought to affect inhibitory and reward functions thought to underlie ADHD. Degradation of these neurotransmitters is performed by the enzymes COMT and MAOA, encoded by the *COMT* gene and *MAOA* gene respectively. *COMT* is highly expressed in the frontal lobe, which is also thought to play an important role in the inhibitory control in ADHD patients. As mentioned above, the functional SNP rs4680, also known as the val/met polymorphism, is most frequently investigated in association with ADHD.

An early small study suggested that the valine allele of rs4680 was associated with ADHD (Eisenberg et al., 1999), the majority of replications report negative results and a meta-analysis suggested no association between ADHD and the val/met polymorphism (Gizer, et al., 2009).

A screen of 23 genes thought to affect ADHD revealed *MAOA* as a particularly promising candidate for the disorder (Guan et al., 2009). This study and an independent candidate gene study suggest association between ADHD and SNP rs12843268, which will be investigated in Chapter Six (Guan, et al., 2009; Rommelse et al., 2008). Several studies suggest that the high activity allele of the *MAOA* confer the risk for ADHD (Gizer, et al., 2009).

1.12.5 IMAGING GENETICS OF ADHD

The field of imaging genetics combines two modalities of psychiatric research in order to find genetic markers for neuroimaging phenotypes associated with disorders. Understanding the genetics behind psychiatric disorders may become easier if the disorder is decomposed into its intermediate phenotypes such as neurocognitive measures. It is believed that fewer genes will contribute to these intermediate phenotypes than to the entire psychiatric disorder (Gottesman & Gould, 2003).

This idea was recently tested in an ADHD sample (Hoogman et al., 2011). The study investigated the effect of Nitric Oxide (*NOS1*) gene on VS activation during reward anticipation. *NOS1* had previously been associated with ADHD in a genome wide association study, and is known to inhibit monoamine transporters, thereby modulating the dopamine and noradrenaline concentration in the brain. Whereas the results suggested that ADHD patients show the expected reduced VS activation during reward anticipation, individuals who carry the ADHD risk genotype of *NOS1* demonstrated higher VS activation than carriers of the other VNTR

genotype. Thus, VS activation during reward anticipation does not appear to mediate the association between *NOS1* and ADHD and further studies are needed to determine the neurocognitive mechanisms thought to underlie ADHD.

Several imaging genetic studies have aimed to determine the impact of dopaminergic genes on reward-related VS activation, without associating the gene-brain relationship with a disorder. A study by Forbes and colleagues suggested that multiple dopamine genes, including *DRD2*, *DAT1* and *DRD4*, explained as much as 12% of the variance in VS activation measured during reward feedback (Forbes et al., 2009). These results were supported in a study by Nikolova and colleagues which suggested that a multilocus genetic profile including *DAT1*, *DRD4*, *DRD2* and *COMT* accounted for 10.9% of the inter-individual variability in VS activation during measured during a card guessing game (during reward feedback) (Nikolova, Ferrell, Manuck, & Hariri, 2011).

Another imaging genetic study targeting the reward system suggested that *COMT* in combination with *DAT1* affects brain activation during reward anticipation (Dreher, Kohn, Kolachana, Weinberger, & Berman, 2009). A gene-gene interaction between *COMT* and *DAT1* was found in the activation of the VS and lateral prefrontal cortex during reward anticipation, with carriers of the *DAT1* 9-repeat allele and *COMT* Met/Met allele exhibiting the highest activation. These results indicate that genetically influenced variations in dopamine transmission modulate the response of brain regions involved in the anticipation of rewards (Dreher, et al., 2009). *DAT1* was also tested in an imaging genetic study investigating the interplay between VS activity during reward anticipation and trait reward sensitivity. The results suggested that homozygote carriers of the *DAT1* 10-repeat allele exhibit a strong positive correlation between reward sensitivity (as indexed by the sensitivity to

punishment and sensitivity to reward questionnaire; SPSRQ) and reward related VS activity whereas this relationship is absent in the *DAT1* 9-repeat allele carriers (Hahn et al., 2011).

Two imaging genetic studies have attempted to investigate the relationship between *MAOA* and fMRI BOLD-responses during tasks targeting emotion and inhibition, two neurocognitive mechanisms thought to affect ADHD (Meyer-Lindenberg et al., 2006). The study identified an association between *MAOA* and activation of the anterior cingulate in men during inhibitory control, but the study only made a hypothetical link to behaviours that may be affected by this association. The second study by Buckholtz and colleagues showed that males carrying the low expression allele of the *MAOA*-VNTR showed reduced functional connectivity between the ventromedial prefrontal cortex and amygdala during a face processing task (Buckholtz et al., 2008). However, the reduction in connectivity was not observed amongst women. Effect of *MAOA* on VS activation has not been tested during reward processing tasks or any other neuroimaging task. Based on these studies *MAOA* may be a candidate gene underlying gender-specific brain function.

Imaging genetics is a powerful approach to investigate the neurobiology of behaviour. However, it has been argued that the true potential of this approach will only be achieved once larger sample sizes are available (Viding, Williamson, & Hariri, 2006). To date, most imaging genetic studies are performed on sample sizes of 30-40 individuals. Authors acknowledge that in order to have the power to determine the associations between genes, neural function and disorders larger sample sizes of well-characterised populations are necessary.

1.13 THESIS OUTLINE

This thesis is composed of 4 empirical chapters aimed to investigate reward processing in adolescence and its relationship with ADHD symptoms. We also investigated genetic variants underlying reward processing in adolescence. All empirical chapters are based on data collected from the IMAGEN sample (Schumann et al., 2010). The IMAGEN sample is the largest adolescent imaging genetic study performed to date. It provides neuropsychological, neuroimaging and genetic data on a sample of 2000 13-15 year old adolescents. Whereas Chapters Three and Four are based on neuroimaging data exclusively, Chapters Five and Six are based on both neuroimaging and genetic data. As such Chapters Three and Four are based on data from the full sample, whereas Chapters Five and Six are based on data from wave 1 of the IMAGEN, as genetic data was not available for the second and third wave at the time of analysis. Below, is a brief overview of the chapters of this thesis.

Chapter 2: Methodology

This chapter outlines the methodology used by the IMAGEN sample, with particular focus on the neuroimaging, behavioural and clinical measures investigated in this thesis.

Chapter 3: Random Effects Analyses of Reward Processing

This chapter investigates a large sample of 1,243 adolescents (584 boys, 659 girls) to determine brain activation patterns associated with reward anticipation and reward feedback trials, measured during the MID Task. To ensure that our data was associated with reward-processing, we only used successful hit-trials and the contrast-maps were controlled for baseline by subtracting all activation associated with

anticipation/feedback no win from the anticipation/feedback high win. This study aimed to determine which regions are activated within the reward system in a sample of 13-15 year old adolescents. Based on prior research we focused particularly on activation patterns within the OFC and VS. Considering that this is the largest study of reward processing to date, we also aimed to determine whether there is any overlap between brain regions activated during reward anticipation and brain regions activated during reward feedback.

Chapter 4: Gender Differences in Reward Processing and the Gender Specific Association between Ventral Striatal Activation and ADHD Symptoms

This chapter explores gender differences in reward processing. We performed whole-brain t-tests on a sample of 1,234 adolescents (579 boys, 655 girls). We aimed to determine whether gender differences appear during both the reward anticipation and the reward feedback stage of reward processing. Furthermore, we investigated whether the activation patterns during reward anticipation and reward feedback differently relate to ADHD symptoms in males and females.

Chapter 5: *MAOA* Genotype Affects Ventral Striatal Activation in Boys, but Not Girls

This chapter provides results from an imaging genetic study of 411 adolescents (186 boys, 225 girls) aimed to determine the effect of the X-linked gene *MAOA* on ventral striatal activation during reward anticipation in boys and girls separately. In addition to the effect of *MAOA* on VS activation we also aimed to determine whether VS activation and *MAOA* genotype affects novelty seeking and impulsivity in adolescence.

Chapter 6: Neural Mechanisms of ADHD Symptoms are Stratified by *MAOA* Genotype

This chapter explores whether the X-linked gene *MAOA* which has been previously associated with ADHD also affects neural mechanisms known to be associated with the disorder. Focusing particularly on the *MAOA* SNP rs12843268 we determined an association with ADHD symptoms in a sample of 190 male adolescents from the first wave of IMAGEN. We also noted that VS activation was negatively correlated with ADHD symptoms amongst the A hemizygotes of rs12843268 and that right IFG activation during successful inhibition was positively correlated with ADHD symptoms amongst the G hemizygotes of rs12843268.

Chapter 7: Conclusions

This chapter summarises the findings presented in the preceding chapters together with a discussion of their implications for clinical practice and future research. A critique of the studies presented in this thesis is also provided in this chapter.

BOX 1: REWARD DEFICIENCY SYNDROME

The Reward Deficiency Syndrome (RDS) results from a dysfunction of the dopaminergic reward system of the brain. The RDS was firstly referred to in research of addictive behaviours – and particularly in studies of alcoholism (Blum, Cull, Braverman, & Comings, 1996; Blum & Noble, 1990). The RDS originated from the association between alcoholism and the dopamine receptor gene *DRD2* (Blum et al., 1996). Dopamine is an important neurotransmitter in the brain's reward system and is known to control moods and feelings of well-being (Delgado, 2007). Individuals who suffer from underactivation (i.e. a deficiency) of the dopaminergic system, often due to genetic predispositions, will engage in activities that increase the activation of the system in order to receive the pleasant stimulation of its activation (Comings & Blum, 2000). Whereas the RDS originated from genetics, it has now come to refer to a dysfunctional state of the reward system independent of any specific genetics (Blum, Cull, et al., 1996; Blum & Noble, 1990; Hommer, et al., 2011). Several reward-related disorders, such as addictions and ADHD, are characterised by deficient activation of brain regions in response to rewards and are thus referred to as reward deficiency disorders (Blum, Cull, et al., 1996). In fact, the RDS has been suggested to explain a number of reward seeking behaviour such as extreme impulsivity and novelty seeking, which are often manifested in the form of antisocial behaviours (Comings & Blum, 2000). The reward deficiency hypothesis may help explain increased risk taking in adolescence as well as reward-related disorders such as ADHD (see ***Table 1*** and ***Table 2***).

BOX 2: IMPULSIVITY HYPOTHESIS

The impulsivity hypothesis is an opposing theory of the RDS hypothesis. It suggests that a combination of excessive reward seeking and failure of effective inhibition underlies novelty seeking and impulsive behaviour. Whereas the RDS hypothesis suggests that novelty seeking and impulsivity are the result of an underactive reward system, the impulsivity hypothesis suggests that these behaviours result from an overactive reward system in combination with insufficient inhibitory control (Ernst, Pine, & Hardin, 2006; Hommer, Bjork, & Gilman, 2011). The impulsivity hypothesis was initially based on the fact that longitudinal studies suggested that individuals who demonstrated poor behavioural self-control or high novelty seeking in childhood were substantially more likely to initiate substance use and other reward seeking behaviours in adolescence and they were also more likely to develop substance dependence in adulthood (Hommer, et al., 2011). Collectively, these studies suggest that reward-related behaviours and disorders are characterised by increased activation of the reward system together with reduced activation of the inhibitory system. The Impulsivity hypothesis may assist our understanding of reward seeking behaviour in adolescence and in individuals with ADHD (see ***Table 1*** and ***Table 2***).

2 CHAPTER TWO:

METHODOLOGY

2.1 OBJECTIVES OF THIS CHAPTER

The data analysed and presented in this thesis was collected as part of the IMAGEN study. This chapter outlines the methodology and research instruments employed in IMAGEN. The specific aims of this chapter are as follows:

1. Provide an overview of the participant characteristics, recruitment and assessment procedures of IMAGEN
2. Provide a detailed account of the psychometric and behavioural research assessment tools employed
3. Provide a detailed account of the functional and structural neuroimaging procedures
4. Provide an account of the genotyping methods adopted by IMAGEN
5. Provide an account of the expression analysis performed to determine expression levels of single nucleotide polymorphisms

2.2 IMAGEN: BACKGROUND, PARTICIPANTS, RECRUITMENT AND PROCEDURES

2.2.1 BACKGROUND

IMAGEN is the first multi-centre, imaging genetics study aimed at identifying genetic and neurobiological factors underlying variability in impulsivity, reinforcer sensitivity and emotional reactivity and determining their predictive value for the development of frequent psychiatric disorders (Schumann, et al., 2010). The study was carried out by the IMAGEN consortium under the lead of Gunter Schumann, who developed the study in 2006. The study is conducted across eight sites located in London, Dublin, Mannheim, Berlin, Nottingham, Paris, Hamburg and Dresden. Comprehensive behavioural and neuropsychological characterisation is performed on healthy adolescents, aged 13-15 years, and followed up at later time points. IMAGEN receives research funding from the European Community's Sixth Framework Programme (LSHM-CT-2007-037286).

2.2.2 PARTICIPANTS

Participants were recruited from secondary schools across the eight study sites. The full IMAGEN sample included > 2,000 adolescents. Data-collection was completed in two waves. The first wave, on which Chapters Five and Six are based, totalled 705 adolescents (mean age: 14.35; SD: 0.44) of which 48.2% were female and 91.8% were Caucasian. The full dataset, on which Chapters Three and Four are based, totalled 2030 adolescents (mean age: 14.55; SD: 0.45) of which 51.4% were female and 87.3% were Caucasian. However, for each individual analysis performed these numbers were reduced based on the number of individuals for whom data was available and whether this data survived stringent quality control measures.

2.2.3 RECRUITMENT

The recruitment procedures were standardised across the eight study sites. Geographical areas were chosen for ethnic homogeneity. All schools within the selected geographical areas were contacted by phone and/or letter. IMAGEN research assistants visited schools to explain the project and to gain permission to recruit from the school. After receiving consent, the team visited the schools to meet with students. IMAGEN participants were recruited during school visits, during which the study was presented and an information pack was given for students to take home. If students had chosen to provide the team with contact details they were called in the evenings or weekends to answer any questions that they or their parents/guardians had about the project. Upon receipt of consent forms, participants were sent information about how to complete the home assessment and a date was arranged for the parent/guardian and child to visit their local centre.

2.2.4 EXCLUSION CRITERIA PRIOR TO ASSESSMENT

Participants were aged 14 years ± 3 months at time of recruitment in order to control for differences in brain development patterns. Participants were excluded prior to assessments if they met the following criteria:

1. Were not able to attend a full assessment day at the local research institute
2. Had contraindications for magnetic resonance imaging, such as braces or other metal implants
3. Were born prematurely
4. Displayed specific illnesses such as epilepsy or diabetes
5. Had experienced head trauma
6. Were taking medication which may affect either function or anatomy of the central nervous system

2.2.5 TESTING PROCEDURES

2.2.5.1 Home Assessment

Two weeks prior to the institute visit, the participant completed a home assessment conducted through the web-based coordination system *Psytools*, which was developed for the purpose of multi-site, multi-lingual assessments (Delosis, London, UK). Participants were provided with instructions for the home assessment, including a unique identification code and an internet link to download the psychometric battery in computerised format. The home assessment included reliability check variables to detect nonsensical and untruthful responding. The assessment also provided checks regarding the working environment. If deemed necessary, participants were asked to repeat tasks at the institute assessment. Data that was deemed unreliable were excluded from further analyses.

2.2.5.2 Institute Assessment

The institute assessment was completed during one or two visits, taking approximately eight hours in total. When the assessment was split over two visits, the visits were separated by no longer than three months. During the institute assessment, participants completed cognitive and behavioural tasks and were instructed on neuroimaging assessment prior to performing two MRI sessions lasting ~45 minutes each. Parents of participants completed tasks regarding the child's personality and behaviour as well as information on their own drinking and smoking habits.

2.2.6 ETHICAL APPROVAL

Ethical approval was obtained from the local ethics committee at each study site. IMAGEN recruited a multi-disciplinary ethics group to develop new strategies for dealing with sensitive issues that may arise from combining genetic, biological and

environmental findings across sites. Prior to participation, full parental consent and participant assent was obtained.

2.3 PSYCHOMETRIC AND BEHAVIOURAL ASSESSMENT

The psychometric and behavioural characterisation of the participants was established using the software program *Psytools* (Delosis, London, UK). Psytools presents questionnaire items and response alternatives on a computer screen on any computer platform, so it was used for both home and institute-based data collection. Participants were instructed to answer by clicking on corresponding virtual response buttons using a computer mouse. There was a version for both the adolescent and the parent or guardian (but parents only completed their tasks during the institute session).

2.3.1 DEMOGRAPHICS

At the start of each task within the adolescent battery, participants were asked for gender, age and school grade. Data on ethnicity was collected as part of a family history questionnaire completed by the parent.

2.3.2 WECHSLER INTELLIGENCE SCALE FOR CHILDREN (WISC)-IV

The Wechsler Intelligence Scale for Children (WISC) is an intelligence scale for children and adolescence between the ages of 6 and 16 years. The adaptation used in IMAGEN focuses on two scales, the Verbal Comprehension Index (VCI) and the Perceptual Reasoning Index (PRI). The VCI is composed of five subscales: i) vocabulary, where the participant is asked to define a word; ii) similarities, where the participant is asked how two words are alike/similar; iii) comprehension, where the participants answers questions about social situations or common concepts; iv) information, composed of general knowledge questions; v) word reasoning, a task

involving clues that lead to a specific word. The PRI is composed of three subscales. i) block design, where participants put together red and white blocks in patterns according to a displayed model; ii) picture concept, where participants are provided with a series of pictures presented in rows and asked to determine which pictures go together; iii) matrix reasoning, where participants are shown an array of pictures with one missing square and select the picture that fits the array.

The reliability of WISC-IV has been extensively tested in a standardization sample of 2,200 children and adolescents. Based on data from this sample, the test shows good internal consistency and test-retest reliability. The validity of WISC-IV has been tested in relation to several other measures including: WISC-III, WAIS-III and WASI and show good correlations (http://media.wiley.com/product_data/excerpt/50/04701891/0470189150.pdf). The WISC-IV has also been adapted and standardised in French and German.

2.3.3 Strengths and Difficulties Questionnaire (SDQ)

ADHD symptoms were assessed using the parental ratings of the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997) (see **Appendix 1**). The SDQ is a 25 item measure that assesses five aspects of behaviour, which can be linked to different psychopathologies: emotional symptoms, conduct problems, hyperactivity/inattention (for the purpose of the thesis, this is called ADHD symptoms), peer-problems and pro-social behaviour. For each item participants (i.e. both the adolescent and their parents) are asked to indicate on a three-point scale the extent to which the statements reflect their own/their child's behaviour over the past six months (0 = not true, 1 = somewhat true, 2 = certainly true). For the purposes of the current analyses, only the ADHD symptoms scale will be investigated. Five items are used to assess ADHD symptoms, that include impulsivity, hyperactivity and

inattention (i.e. ‘I am constantly fidgeting or squirming’; ‘I am easily distracted, I find it difficult to concentrate’; ‘I think before I do things’; ‘I finish the work I'm doing. My attention is good’; ‘I am restless, I cannot stay still for long’). The current study used parental reports on the SDQ as externalising problems in children have been shown to be more reliably measured by parents than by self-report (Herjanic & Reich, 1997). Based on ratings on the ADHD symptoms scale individuals are also given a likelihood-rating as being a ‘possible’ or ‘probable’ case of ADHD. No participant in IMAGEN was rated as a ‘probable’ case of ADHD and only 91 individuals (out of the 1,243 who survived neuroimaging quality control criteria) were rated as ‘possible’ cases.

The SDQ is a reliable and valid measure of youth emotional and behaviour symptoms, on which extreme scores are predictive of increased probability of clinician-rated psychiatric disorders and retest stability over 4-6 months (Goodman, 2001). German and French versions of the SDQ exist and preliminary research suggests that these translated versions have similar internal structure to the English version (Woerner et al., 2002). The SDQ is suitable for use with adolescents aged 11 to 16 years and has been shown to be a reliable and well validated measure of adolescent emotional and behavioural symptoms (Goodman, 2001). This ADHD symptoms subscale has been validated and has been associated with ADHD diagnosis according to DSM-IV (Goodman, 2001). Scores for the five ADHD symptoms subscales were combined to create a composite ADHD symptoms total score.

2.3.4 TEMPERAMENT AND CHARACTER INVENTORY (TCI)

Novelty Seeking was assessed using self-ratings of the Novelty Seeking Scale of the Cloninger’s Temperament and Character Inventory-Revised Version (TCI-R; Cloninger et al. 1999) (see **Appendix 2**). The TCI-R Novelty Seeking Scale is a 34-

item scale that measures 4 aspects of personality: i) Exploratory Excitability vs. Rigidity; ii) Impulsiveness vs. Reflection; iii) Extravagance vs. Reserve; iv) Disorderliness vs. Regimentation. For each item participants are asked to indicate on a five-point scale the extent to which the statements reflect their own behaviour over the past six months (1 = definitely false, 2 = mostly false, 3 = neither true or false, 4 = mostly true, 5 = definitely true). Twelve items have reversed coding.

The Novelty Seeking Scale of TCI-R is a reliable measure of youth novelty seeking and impulsivity (Cloninger, Bayon, & Svrakic, 1998; de la Rie, Duijsens, & Cloninger, 1998). German and French translation of the TCI-R are available and preliminary research suggests good reliability in these international translations across clinical and non-clinical subject groups (Pelissolo et al., 2005; Snopek, Hublova, Porubanova, & Blatny, 2012).

2.3.5 PUBERTY DEVELOPMENT SCALE (PDS)

We administered the Puberty Development Scale (PDS; Peterson et al., 1988) to reliably assess the pubertal status of our adolescent sample. This scale provides an eight-item self-report measure of physical development based on the Tanner stages with separate forms for males and females (see **Appendix 3**). For this scale, there are five categories of pubertal state: i) pre-pubertal, ii) early pubertal, iii) mid-pubertal, iv) late pubertal, v) post-pubertal. Participants answered questions regarding their growth in stature and pubic hair, as well as menarche in females and voice changes in males. Dorn et al (1990) compared self-ratings and physician ratings of pubertal development and found significant correlations between adolescent self-rating and physician's rating (for males: r=.77 to r=.84, females: r=.88 to r=.91).

2.4 NEUROIMAGING ASSESSMENT

2.4.1 THE BOLD-RESPONSE

Neuroscientists have used the non-invasive method of functional magnetic resonance imaging (fMRI) to investigate activation patterns in the human brain by observing changes in blood flow. The most commonly used form of fMRI measures brain activation through the blood oxygen level-dependent (BOLD) response which is an indirect measure of neural activation in the brain by the measurement of oxygenated blood vs. non-oxygenated blood in a particular region. The BOLD response measures the change in magnetization in oxygen-rich blood compared to oxygen-poor blood in the brain. Oxygen-poor blood is more magnetic than oxygen-rich blood, which is virtually nonmagnetic. Due to the magnetic properties of oxygen-rich blood molecules spin at a low rate when in a magnetic field whereas the molecules within oxygen-poor blood will spin at a much higher rate.

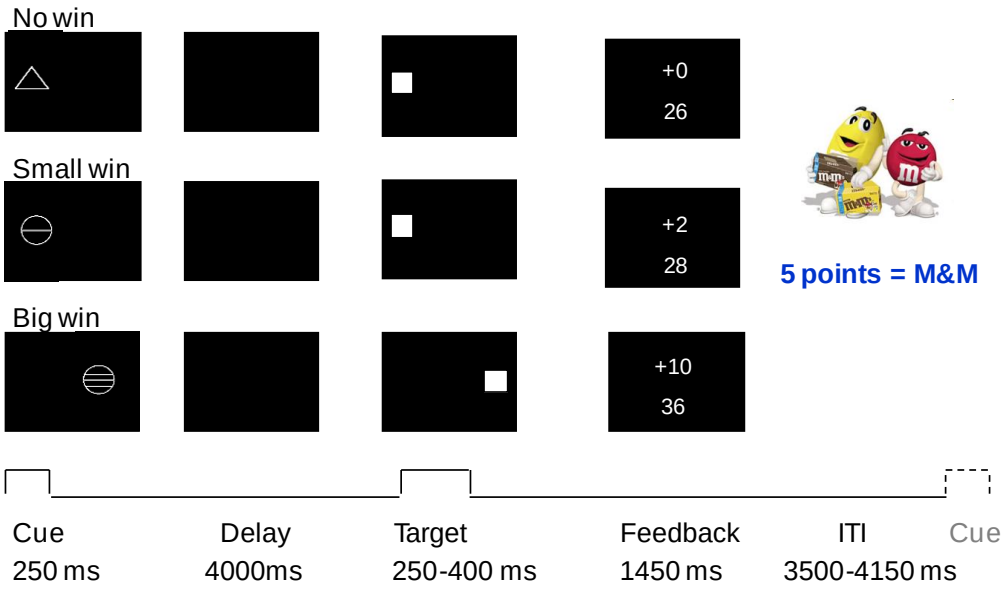
Thus, functional MRI measures changes in blood flow rather than neural activation as such, but blood flow is believed to be associated with neural activation in the brain. When neurons in the brain are activated, blood flow to that region increases so that oxygen-rich blood displaces oxygen-poor blood around two seconds following the activation. A peak in blood flow will appear 4-6 seconds thereafter before returning to the original state. Using functional MRI we are able to measure activation-patterns associated with many cognitive processes. Here, we particularly focus on measuring activation in the reward system.

2.4.2 MONETARY INCENTIVE DELAY (MID) TASK

The participants performed a modified version of the Monetary Incentive Delay (MID) task to study neural responses to reward anticipation and reward feedback (Knutson, Fong, et al., 2001). This event-related task consisted of 66 10-second trials.

In each particular trial, participants were presented with one of three cue shapes (cue, 250 ms) denoting whether a target (a white square) would subsequently appear on the left or right side of the screen and whether 0, 2 or 10 points could be won in that particular trial (***Figure 1***). After a variable delay (4,000-4,500 ms) of fixation on a white crosshair, participants were instructed to respond by pressing a button with their left or right index finger as soon as the target appeared. Feedback on whether and how many points were won during the trial was presented for 1,450 ms after the response. Using a tracking algorithm, task difficulty (i.e. target duration varied between 250 and 400 ms) was individually adjusted such that each participant successfully responded on ~66% of trials. Participants had first completed a practice session outside the scanner (for ~5 minutes), during which they were instructed that for each 5 points won they would receive one food snack in the form of small chocolate candies. Functional MRI BOLD-responses were measured during reward anticipation and reward feedback. The current study used the contrast 'anticipation high win vs. no win' and 'feedback high win vs. no win'. Only successfully 'hit' trials were included for analysis.

Figure 1. Outline of the stages of the MID task



2.4.3 STOP SIGNAL TASK (SST)

Participants also performed an event-related stop signal task (SST) task designed to study neural responses to successful and unsuccessful inhibitory control (Rubia, et al., 2005; Rubia, et al., 2007). The task was composed of Go trials and Stop trials. During Go trials (83%; 400 trials) participants were presented with arrows pointing either to the left or to the right. During these trials subjects were instructed to make a button response with their left or right index finger corresponding to the direction of the arrow. In the unpredictable Stop trials (17%; 80 trials), the arrows pointing left or right were followed (on average 300 ms later) by arrows pointing upwards; participants were instructed to inhibit their motor responses during these trials. A tracking algorithm changes the time interval between Go signal and Stop signal onsets according to each subject's performance on previous trials (average percentage of inhibition over previous Stop trials, recalculated after each Stop trial), resulting in 50% successful and 50% unsuccessful inhibition trials. The inter-trial interval was 1,800 ms. The tracking algorithm of the task ensured that subjects were successful on 50% of Stop trials and worked at the edge of their own inhibitory capacity. The current study only analysed the contrast 'successful Stop trials vs. successful Go trials'. The dependent variable of the task is the stop signal reaction time (SSRT) calculated by subtracting the mean stop signal delay (SSD: the average time between Go and Stop signal, at which the subject managed to inhibit to 50% of trials) from the mean reaction time (MRT) to Go trials, i.e. MRT-SSD (Logan, Schachar, & Tannock, 1997).

2.4.4 NEUROIMAGING ACQUISITION AND ANALYSIS

Structural and functional MRI data were acquired at eight IMAGEN assessment sites with 3T MRI scanners of different manufacturers (Siemens, Philips, General Electric,

Bruker). The scanning variables were specifically chosen to be compatible with all scanners. The same scanning protocol was used in all sites. In brief, high-resolution T1-weighted 3D structural images were acquired for anatomical localization and co-registration with the functional time-series. Blood oxygen-level dependent (BOLD) functional images were acquired with a gradient-echo, echo-planar imaging (EPI) sequence. For the MID task, 300 volumes were acquired for each subject. For the SST, 444 volumes were acquired for each subject. For both tasks, each volume consisted of 40 slices aligned to the anterior commission/posterior commission line (2.4mm slice thickness, 1mm gap). The echo-time was optimised (TE=30ms, TR=2.2s) to provide reliable imaging of subcortical areas.

Functional MRI data were analysed using SPM-8 (Statistical Parametric Mapping, 8th edition, http://www.fil.ion.ucl.ac.uk/spm). Slice-time correction was conducted to adjust for time differences due to multislice imaging acquisition, all volumes were aligned to the first volume and non-linear warping was performed to an EPI template. Images were then smoothed with a Gaussian kernel of 5-mm full-width at half-maximum.

At the first level of analysis, changes in the BOLD-response for each subject were assessed by linear combinations at the individual subject level, for each experimental condition, each trial (i.e. reward anticipation high win) was convolved with the hemodynamic response function to form regressors that account for potential noise variance associated with the processing of reward anticipation and reward feedback. Estimated movement parameters were added to the design matrix in the form of 18 additional columns (3 translations, 3 rotations, 3 quadratic and 3 cubic translations, and each 3 translations with a shift of ±1 TR). See **Appendix 4** for first level models of the MID and SST as created by Neurospin. To analyse the

anticipation phase we contrasted 'anticipation of high win [here signalled by a circle] vs. anticipation of no win [here signalled by a triangle]' and to analyse the feedback phase we contrasted 'feedback of high win vs. feedback of no win'. To analyse successful inhibition we contrasted 'successful Stop trials vs. successful Go trials'. Single-subject contrast images were normalised to Montreal Neurological Institute (MNI) space. The normalised and smoothed single-subject contrast images were then taken to a second-level random effects analysis. ROIs were extracted using the Marsbar toolbox (http://marsbar.sourceforge.net). The mask images (mask.img) produced by the second level analysis for each contrast are available in **Appendix 5**.

2.5 GENOTYPING METHOD

Blood samples were collected at the local institute and sent to the DNA bank at regular intervals for processing to allow analyses of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein and immortalised B cells.

DNA purification and genotyping was performed by the Centre National de Génotypage in Paris. DNA was purified from whole blood samples (~10ml) preserved in BD Vacutainer EDTA tubes (Becton, Dickinson and Company) using Gentra Puregene Blood Kit (Qiagen) according to the manufacturer's instructions. Genotype information was collected at 582,982 markers using the Illumina HumanHap610 Genotyping BeadChip.

Single nucleotide polymorphisms (SNPs) with call rates of < 98%, minor allele frequency < 1% or deviation from the Hardy-Weinberg equilibrium ($p \leq 1\times10^{-4}$) were excluded from the analyses. Individuals with an ambiguous sex code, excessive missing genotypes (failure rate > 2%), and outlying heterozygosity (heterozygosity rate 3 standard deviations from the mean) were also excluded. Identity-by-state similarity was used to estimate cryptic relatedness for individual

using PLINK software. Closely related individuals with Identity-by-descent (IBD > 0.1875) were eliminated from the subsequent analysis. Population stratification for the GWAS data was examined by principal component analysis (PCA) using EIGENSTRAT software. The four HapMap populations were used as reference groups in the PCA and individuals with divergent ancestry (from CEU) were also excluded.

2.6 EXPRESSION ANALYSIS

We were particularly interested in measuring expression levels of the gene Monoamine Oxidase A (*MAOA*). Total RNA was extracted from whole blood cells using the PAXgene Blood RNA Kit (QIAGEN Inc., Valencia, CA, USA). Following quality control of the total RNA extracted, labelled complementary RNA (cRNA) was generated using the Illumina® TotalPrep™ RNA Amplification kit (Applied Biosystems/Ambion, Austin, TX, USA). Complementary RNA was purified and quantified using a Qubit® 2.0 Fluorometer (Invitrogen, Paisly, UK). The size distributions of cRNA was determined through Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) using the Eukaryotic mRNA Assay with smear analysis. Gene expression profiling was performed using Illumina HumanHT-12 v4 Expression BeadChips (Illumina Inc., San Diego, CA, USA). Expression data was normalised using the *mloess* method (Sasik, Calvo, & Corbeil, 2002). Expression data for probes mapping to *MAOA* was extracted and tested for association with *MAOA* genotype.

As a significant association was identified between *MAOA* genotype and gene expression in boys, *MAOA* gene expression was independently measured via quantitative polymerase chain reaction (qPCR). Complementary DNA was first synthesised from 40 RNA samples (20 of each genotype of the *MAOA* polymorphism rs12843268) using the SuperScript® III First-Strand Synthesis superMix for

quantitative real time PCR kit (Invitrogen, Paisley, UK) according to the manufacturer's instructions. Secondly, qPCR was performed on cDNA samples in triplicate using the *MAOA* TaqMan® probes (Hs02383327_s1 and Hs01019655_m1, mapping different isoforms of the gene) and the ribosomal *18S* housekeeping probe (Hs 99999901_s1) (Applied BioSystems, Paisley, UK) on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Paisley, UK). Finally, the relative fold change in expression was measured via the comparative method using the formula $2^{-\Delta\Delta Ct}$.

2.7 EXCLUSION CRITERIA PRIOR TO ANALYSES

In addition to the exclusion criteria presented in **Section 2.2.4**, participants were excluded after assessment but prior to analyses if they met the following criteria:

1. Had not completed the structural MRI and/or functional MRI tasks
2. Moved more than 3 mm or 3 degrees in any direction
3. Showed outlying activation values across voxels during the contrast investigated
4. Showed structural abnormalities
5. Reported that they had problems reading the instructions during the functional MRI task investigated or reported falling asleep during the MRI assessment
6. Had a verbal or performance IQ of less than 75
7. Lacked IQ scores, handedness information or questionnaire data on the SDQ or TCI depending on which measure was investigated

In Chapters Five and Six participants were also excluded if they had not survived quality control assessment of the genetic data.

3 CHAPTER THREE:

RANDOM EFFECT ANALYSES OF REWARD PROCESSING MEASURED BY THE MONETARY INCENTIVE DELAY TASK IN ADOLESCENTS

3.1 OBJECTIVES OF THIS CHAPTER

In this chapter random effect analyses of brain activation patterns associated with reward processing in a large adolescent population are presented. Two phases of reward processing were examined: the reward anticipation phase and the reward feedback phase. The specific aims of this chapter are as follows:

1. Determine whether the ventral striatum (VS) is activated during the contrast 'reward anticipation high win vs. reward anticipation no win' and/or the contrast 'reward feedback high win vs. reward feedback no win'
2. Determine whether the orbitofrontal cortex (OFC) is activated during the contrast 'reward anticipation high win vs. reward anticipation no win' and/or the contrast 'reward feedback high win vs. reward feedback no win'
3. Explore whether other brain regions are activated during 'reward anticipation high win vs. reward anticipation no win' and 'reward feedback high win vs. reward feedback no win'
4. Compare results from this study to results from a meta-analysis of reward processing
5. Investigate whether there is an overlap between brain regions activated during reward anticipation and reward feedback

3.2 INTRODUCTION

Adolescence represents a time in development when the brain's reward system undergoes substantial changes (Chambers, et al., 2003; Spear, 2000). Abnormalities in reward processing also underlie many reward-related psychiatric disorders (e.g. addictions and antisocial behaviours), which emerge in adolescence (Breslau, Miller, Chung, & Schweitzer, 2011; Zimic & Jukic, 2012). In order to understand whether changes to the reward system make adolescents more vulnerable to the development of psychiatric disorders, it is important to first characterise reward processing in typically developing adolescents. In this chapter we aim to characterise typical brain activation patterns during two phases of reward processing. In order to do so we use the Monetary Incentive Delay (MID) task.

The neural mechanisms underlying reward processing in primates were uncovered using single cell recordings of macaque monkeys (see **Section 1.3**). In these experiments, Schultz and colleagues showed that reward processing is often composed of two phases; in the first phase rewards are predicted or anticipated and in the second phase rewards are received or consumed (Schultz, et al., 1997). Based on these findings, Knutson and colleagues designed the MID task (Knutson, et al., 2000). The MID task is designed to measure brain activations while a person anticipates making a simple motor response in order to win a reward. The task also allows for measurement of brain activations during reward consumption. The MID task has become a popular functional MRI task for reward processing. It is believed that BOLD-responses in subcortical regions, frequently observed during the MID task, reflect dopaminergic affinity in these regions (Schott, et al., 2008; Schultz, et al., 1997). In fact, the VS is the main receiver of dopaminergic inputs from the ventral

tegmental area (VTA) and tends to show high activation during functional MRI tasks investigating reward processing.

The reward system has not been investigated longitudinally using the same subject at various ages, thus, we know little about its development. However, some studies have compared reward processing in adolescence to reward processing in adulthood. These studies suggest that adolescents process rewards differently compared to adults, in particular both anticipation and reward feedback appear deficient in adolescents compared to adults (Bjork, et al., 2004; Casey, Getz, & Galvan, 2008; Galvan, 2010). Considering that adolescence is a critical stage of brain development, during which many reward-related disorders such as addiction and antisocial behaviours emerge it is important to gain a better understanding of reward processing across development.

In order to investigate typical and atypical reward processing in humans we need to ensure robust activation in brain regions in response to a particular task. Functional MRI studies which use the MID task to activate the reward system usually activate the VS to some extent. However, most functional MRI-studies are based on small sample sizes (n=20-40) (Knutson, Adams, et al., 2001; Knutson, et al., 2000; Knutson & Cooper, 2005). Authors acknowledge that larger sample sizes are needed in order to robustly visualise the complete reward system. In order to overcome the problem of sample size a meta-analysis attempted to pool existing studies in order to examine the core reward networks in the human brain (Liu, et al., 2011). The meta-analysis aimed to identify common and distinct networks during stages of reward processing, namely reward anticipation and reward feedback. The results supported previous research suggesting that the VS and OFC responded to general reward processing, regardless of temporal stage or valence (Knutson, Adams, et al., 2001;

Knutson, et al., 2000; Knutson & Cooper, 2005). However, the VS was implicated during both stages of reward processing whereas the medial part of the OFC was suggested to be more tuned to reward receipt, suggesting that this area monitors and evaluates reward outcomes. The VS and OFC are also the main projection areas of two distinct dopaminergic pathways, the mesolimbic and mesocortical pathways.

The MID task has been used in several studies to directly examine reward sensitivity in psychiatric disorders. In this study we used random effects analyses of the 'anticipation large win vs. anticipation no win' contrast and the 'feedback large win vs. feedback no win' contrast of the MID task in a sample of 13-15 year old adolescents (n = 1,243) from the IMAGEN study. Based on prior literature, we hypothesised that the VS would be significantly activated during both contrasts. Furthermore, we expected greater activation of the VS during reward anticipation than during reward feedback. We expected greater activation of the OFC during reward feedback compared to reward anticipation. In order to comprehensively characterise activation patterns in the adolescent reward system we performed random effects analyses of the reward anticipation contrast and reward feedback contrast.

3.3 MATERIAL AND METHODS

3.3.1 PARTICIPANTS

We used data from the full sample of IMAGEN (n = 2,030). Individuals who had complete data of the anticipation and outcome phase of the MID task (n = 1,860), passed task specific outlier criteria, particularly in terms of movement (n = 1,623), passed contrast-specific outlier criteria in terms of spike detection control (n = 1,384), had been able to see the task in the scanner (n = 1,374), had complete handedness data (n = 1,364), had complete IQ data (> 75) (n = 1,256) and did not show structural

abnormalities (n = 1,243) were included in the dataset. Thus, 1,243 adolescents passed the criteria (n = 584 boys, n = 659 girls). The sample had a mean age of 14.4 years (SD: 0.4; range: 13.2-15.7 years) (see ***Table 3*** for demographics). Participants were tested in eight IMAGEN assessment centres (London, Nottingham, Dublin, Mannheim, Berlin, Hamburg, Paris & Dresden). The study was approved by local ethics research committees at each site. A detailed description of recruitment and assessment procedures, as well as in/exclusion criteria can be found elsewhere (Schumann, et al., 2010). One thousand and ninety one participants were right-handed and 152 participants were left-handed or ambidextrous. Individuals with verbal or performance IQ < 75 were removed from further analysis. Handedness and study site were controlled for in all analyses.

Table 3. Demographics for total sample (n = 1,243): Means, standard deviations (SD) and ranges are presented as well as ratios for gender and handedness.

Demographics	***Statistics*** ***Mean ± SD (Range)***
Age (years)	14.4 ± 0.4 (*13.2-15.7*)
VIQ	112.2 ± 14.6 (*76-155*)
PIQ	109.5 ± 13.7 (*76-147*)
Gender (F:M)	53:47
Handedness (L:R)	12:88

VIQ: Verbal IQ, PIQ: Performance IQ, F: Female, M: Male, L: Left, R: Right

3.3.2 MONETARY INCENTIVE DELAY (MID) TASK

The participants performed a modified version of the MID task to study neural responses to reward anticipation and reward feedback. The paradigm has been described in a previous publications (Nees et al., 2012)(or see **Section 2.4.1**).

3.3.3 FMRI DATA ACQUISITION AND ANALYSIS

Structural and functional MRI data were acquired at eight IMAGEN assessment sites with 3T MRI scanners of different manufacturers as described in **Section 2.4.3**. In the second level analysis (SPM-design: one-sample t-test) of anticipation large win vs. no win and feedback large win vs. no win the following covariates were added to the second-level model: dummy-coded centre effects for the eight centres, handedness (right/ambidextrous) and gender (see ***Figure 2*** and ***Figure 3***). To determine the overlap of activation patterns in the VS and OFC during reward anticipation and reward feedback, masks were created using WFU Pickatlas (Maldjian, Laurienti, & Burdette, 2004; Maldjian, Laurienti, Kraft, & Burdette, 2003). The mask for the VS was based on (Yacubian et al., 2006) (xyz = ±15, 9, -9, radius of 9 mm) (http://marsbar.sourceforge.net) whereas the mask for the OFC was based on the Automated Anatomical Labelling (AALs) (Tzourio-Mazoyer et al., 2002).

3.4 RESULTS

3.4.1 RANDOM EFFECTS ANALYSIS

3.4.1.1 Anticipation high win vs. Anticipation no win

Random effects analyses revealed widespread activations extending from the striatum during the anticipation high win vs. no win contrast. The peak of activation during reward anticipation appeared in the VS, at ± 9 11 -2 ($p_{FWE\text{-}corrected} < 0.05$). The cluster was very large (k > 47,465 voxels) and extended to the prefrontal and middle frontal

cortex as well as to the parietal and occipital lobes. In order to determine which regions of the brain were activated by this contrast, we overlaid a mask created from the random effects analysis with the AALs available in Marsbar (http://marsbar.sourceforge.net) (see ***Table 4***). This revealed activations during anticipation high win compared to low win in key reward-regions previously identified in the literature. These include the caudate, putamen, pallidum and thalamus, but also the insula and cingulate gyrus, the inferior frontal opercularis and orbitalis and superior and medial frontal regions. Visual regions of the occipital cortex were also significantly activated during anticipation high win vs. no win. Significant BOLD-responses were also seen in premotor regions of the precentral gyrus, including the supplementary motor area, and postcentral gyrus. A significant BOLD-response was also seen in the parietal lobe during anticipation high win vs. no win (***Figure 2*** and ***Table 4***).

Figure 2. Second level model of anticipation large win vs. no win with 9 regressors (dummy-coded sites, handedness and gender) and the resulting brain activation in response to this contrast ($p_{FWE\text{-}corrected} < 0.05$, n = 1,243).

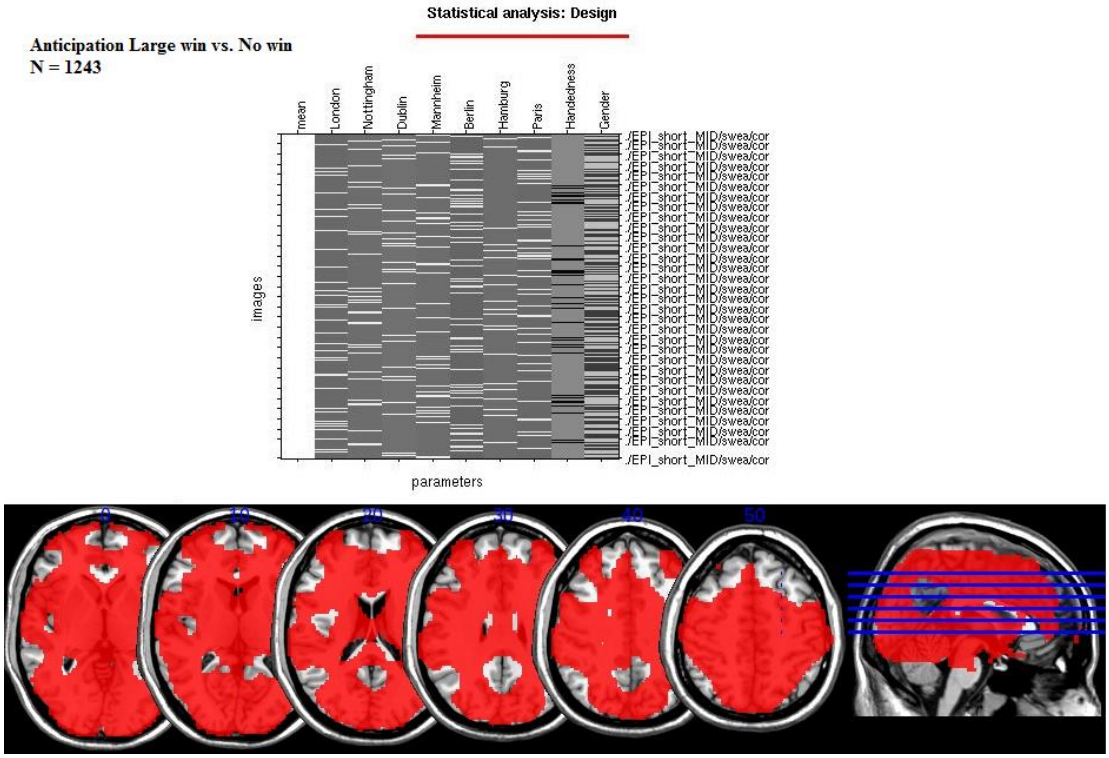


Table 4. Significant brain activation during reward anticipation and reward feedback contrasts ($p_{FWE\text{-}corrected} < 0.05$).

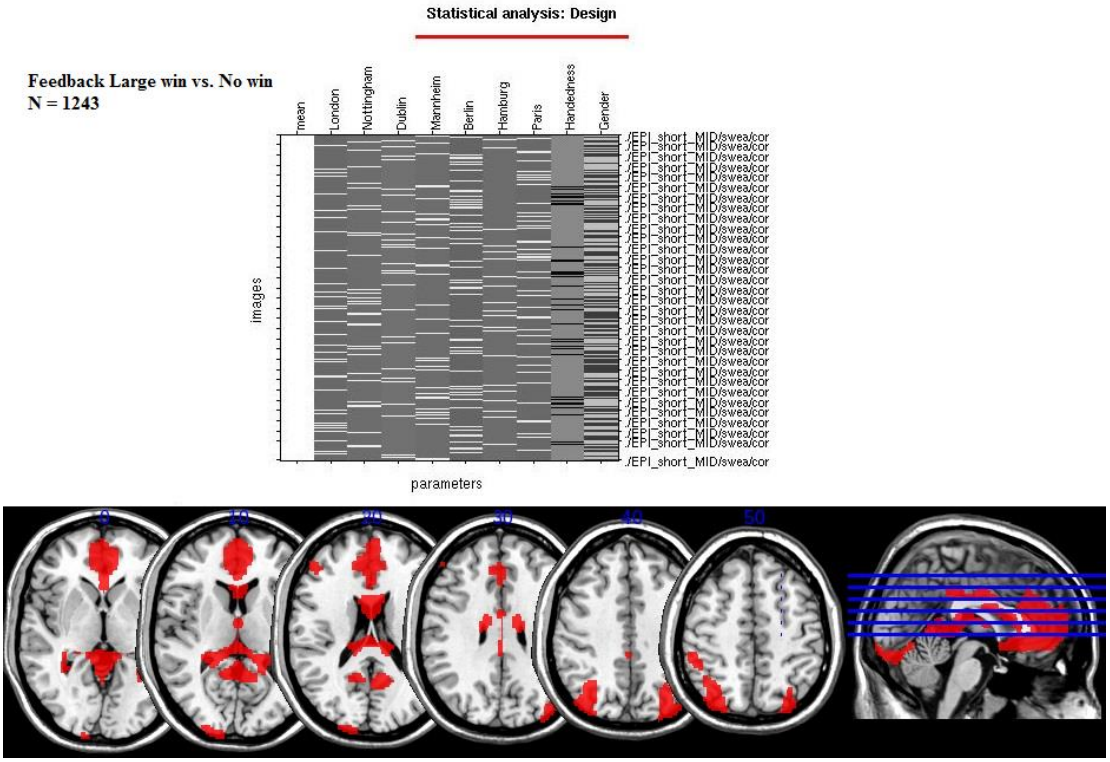
Region	***MNI-coordinates***	***T***	***Cluster size (k)***	$P_{FWE\text{-}corrected}$
Anticipation				
L/R Ventral Striatum	±9 11 -2	42.15	47,465	<0.0001
Feedback				
Anterior Cingulate	3 8 19	26.21	1,438	<0.0001
Medial Orbitofrontal Cortex	0 41 7			
Posterior Cingulate	0 -40 1	18.61	1,896	<0.0001
Parahippocampal gyrus	18 -25 -14			
Thalamus	0 -25 16			
Superior Parietal Lobule	33 -67 46	12.66	531	<0.0001
Precuneus	-30 -70 43	11.75	260	<0.0001
Occipital Pole	18 -103 13	10.32	116	<0.0001

3.4.1.2 Feedback high win vs. Feedback no win

During the feedback high win vs. no win contrast we observed activations in the subcortical regions of the thalamus (x,y,z: 0, -25, 16; $p_{FWE\text{-}corrected} < 0.001$), parahippocampal gyrus (x,y,z: 18, -25, -14; $p_{FWE\text{-}corrected} < 0.001$) and the anterior cingulate gyrus (x,y,z: 3, 8, 19; $p_{FWE\text{-}corrected} < 0.001$). Consistent with previous studies of reward processing we found that the medial OFC was active during reward feedback (x,y,z: 0, 41, 7; $p_{FWE\text{-}corrected} < 0.001$) (Knutson, Fong, et al., 2001; Liu, et al., 2011). We found a significantly higher BOLD-response of the superior parietal lobe during feedback high win trials relative to no win trials (x,y,z: 33, -67, 46; $p_{FWE\text{-}}$

$_{corrected}$ < 0.001). Finally, we found significant activation of the precuneus (x,y,z: -30, -70, 43; $p_{FWE\text{-}corrected}$ < 0.001) and the occipital lobe (x,y,z: 18, -103, 13; $p_{FWE\text{-}corrected}$ < 0.001) (***Figure 3*** and ***Table 5***).

Figure 3. Second level model of feedback large win vs. no win with 9 regressors (dummy-coded sites, handedness and gender) and the resulting brain activation in response to this contrast ($p_{FWE\text{-}corrected}$ < 0.05, n = 1,243).



3.4.1.3 <u>*Overlap between Anticipation contrast and Feedback contrast*</u>

Prior research suggests that the VS is active during both phases of reward processing whereas the OFC is preferentially activated during reward feedback. In this adolescent sample, we aimed to determine whether activation in the VS and OFC are specific to one or both phases of reward processing. We also wanted to determine whether there was an overlap between activation patterns during reward anticipation and reward feedback (see ***Table 5***).

We found that the VS is activated during reward anticipation, but not during reward feedback (***Figure 4***). The OFC is activated during both stages of reward processing. However, there is no overlap between the activation patterns (***Figure 5***). During reward anticipation the activation is centered in the middle OFC. This shifts during the reward feedback phase to the medial OFC.

Using the AALs we also aimed to determine in which regions there is an overlap between the activation patterns of the two contrasts (see ***Table 5***). We found that even in cases when the contrasts activate the same region, the activations rarely overlap. Overlaps between BOLD-responses of reward anticipation and the reward feedback contrasts were observed in the anterior and middle cingulate gyrus. The anterior cingulate gyrus is thought to receive inputs from the thalamus, activated during reward anticipation, and to project to the OFC where we observe activations during the feedback contrast. The superior parietal lobule showed large activations during reward anticipation, but also some activation during reward feedback. The parietal lobule has been associated with the valuation of different option and integration of information. Therefore, it is crucial for the parietal lobule to be involved in both stages of reward processing so as to plan and prepare for an informed action.

Figure 4. Activation patterns in the VS during reward anticipation and reward feedback: Significant activation in the VS was observed during reward anticipation (shown in red). No significant BOLD-response was identified in the VS during reward feedback.

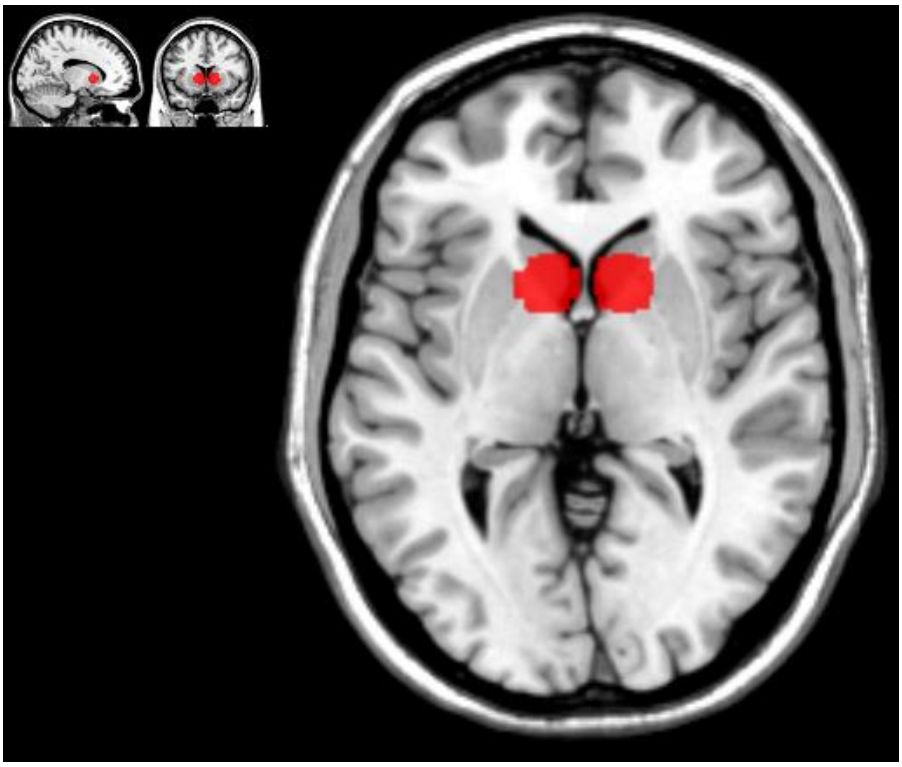


Figure 5. Activation patterns in the OFC during reward anticipation and reward feedback: Significant activation of the OFC was observed during reward anticipation (shown in red; middle OFC) and reward feedback (shown in blue; medial OFC).

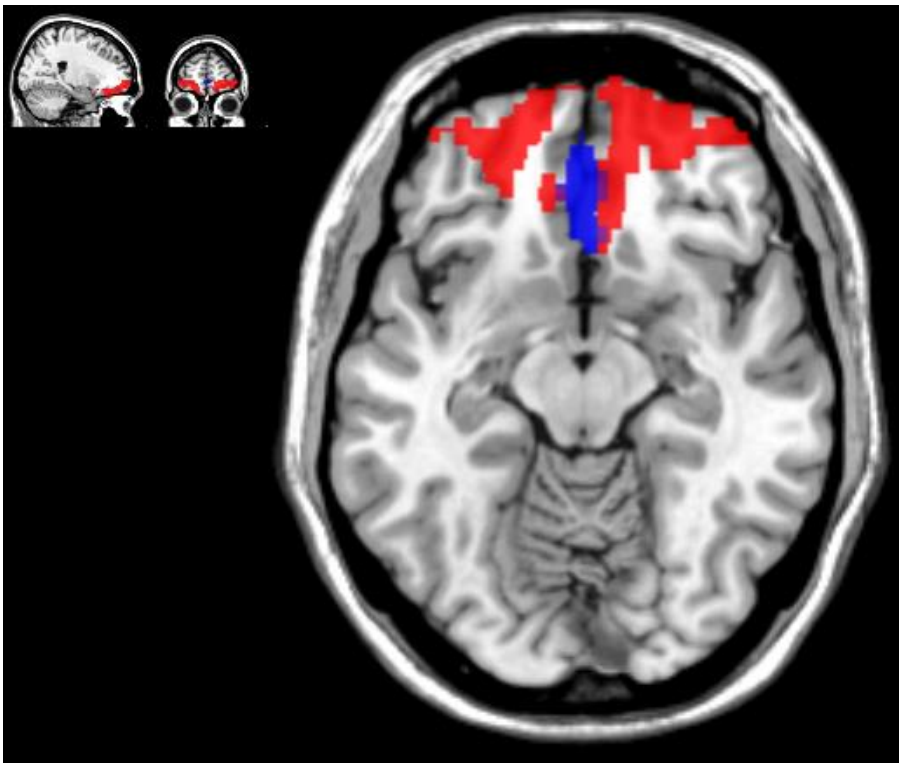


Table 5. Activated Autamatic Anatomical Labeling (AALs) during reward anticipation and reward feedback, and overlap between contrasts

AAL Regions	***Activation during Reward Anticipation?***	***Activation during Reward Feedback?***	***Overlap between Activation during Reward Anticipation and Reward Feedback?***
Left Amygdala	Yes	No	No
Right Amygdala	Yes	No	No
Left Angular Gyrus	No	No	No
Right Angular Gyrus	No	No	No
Left Calcarine	Yes	No	No
Right Calcarine	Yes	No	No
Left Caudate	Yes	No	No
Right Caudate	Yes	No	No
Left Cerebellum	Yes	No	No
Right Cerebellum	Yes	No	No
Left Anterior Cingulate	Yes	Yes	Yes
Right Anterior Cingulate	Yes	Yes	Yes

AAL Regions	*Activation during Reward Anticipation?*	*Activation during Reward Feedback?*	*Overlap between Activation during Reward Anticipation and Reward Feedback?*
Left Mid Cingulate	Yes	Yes	Yes
Right Mid Cingulate	Yes	Yes	Yes
Left Post Cingulate	Yes	Yes	No
Right Post Cingulate	Yes	Yes	No
Left Cuneus	Yes	No	No
Right Cuneus	Yes	No	No
Left Inferior Frontal Opercularis	Yes	No	No
Right Inferior Frontal Opercularis	Yes	No	No
Left Inferior Frontal Orbitalis	Yes	No	No
Right Inferior Frontal Orbitalis	Yes	No	No
Left Inferior Frontal Triangularis	No	No	No
Right Inferior Frontal Triangularis	No	No	No
Left Medial Frontal Orbitalis	No	Yes	No

AAL Regions	*Activation during Reward Anticipation?*	*Activation during Reward Feedback?*	*Overlap between Activation during Reward Anticipation and Reward Feedback?*
Right Medial Frontal Orbitalis	No	Yes	No
Left Middle Frontal Orbitalis	Yes	No	No
Right Middle Frontal Orbitalis	Yes	No	No
Left Medial Frontal Superior	Yes	Yes	Yes
Right Medial Frontal Superior	Yes	Yes	Yes
Left Superior Frontal Orbitalis	Yes	No	No
Right Superior Frontal Orbitalis	Yes	No	No
Left Fusiform	Yes	No	No
Right Fusiform	Yes	No	No
Left Heschl Gyrus	Yes	No	No
Right Heschl Gyrus	Yes	No	No
Left Hippocampus	Yes	Yes	Yes
Right Hippocampus	Yes	Yes	Yes

AAL Regions	*Activation during Reward Anticipation?*	*Activation during Reward Feedback?*	*Overlap between Activation during Reward Anticipation and Reward Feedback?*
Left Insula	Yes	No	No
Right Insula	Yes	No	No
Left Lingual Gyrus	Yes	No	No
Right Lingual Gyrus	Yes	No	No
Left Occipital Cortex	Yes	Yes	Yes
Right Occipital Cortex	Yes	Yes	Yes
Left Olfactory	Yes	Yes	No
Right Olfactory	Yes	Yes	No
Left Pallidum	Yes	No	No
Right Pallidum	Yes	No	No
Left Parahippocampus	Yes	No	No
Right Parahippocampus	Yes	No	No
Left Paracentral Lobule	Yes	No	No

AAL Regions	*Activation during Reward Anticipation?*	*Activation during Reward Feedback?*	*Overlap between Activation during Reward Anticipation and Reward Feedback?*
Right Paracentral Lobule	Yes	No	No
Left Inferior Parietal Lobule	Yes	No	No
Right Inferior Parietal Lobule	Yes	No	No
Left Superior Parietal Lobule	Yes	Yes	Yes
Right Superior Parietal Lobule	Yes	Yes	Yes
Left Postcentral Gyrus	Yes	No	No
Right Postcentral Gyrus	Yes	No	No
Left Precentral Gyrus	Yes	No	No
Right Precentral Gyrus	Yes	No	No
Left Precuneus	Yes	No	No
Right Precuneus	Yes	No	No
Left Putamen	Yes	No	No
Right Putamen	Yes	No	No

AAL Regions	*Activation during Reward Anticipation?*	*Activation during Reward Feedback?*	*Overlap between Activation during Reward Anticipation and Reward Feedback?*
Left Supplementary Motor Area	Yes	No	No
Right Supplementary Motor Area	Yes	No	No
Left Temporal Lobule	Yes	No	No
Right Temporal Lobule	Yes	No	No
Left Temporal Pole	Yes	No	No
Right Temporal Pole	Yes	No	No
Left Thalamus	Yes	Yes	No
Right Thalamus	Yes	Yes	No
Left Vermis	Yes	Yes	No
Right Vermis	Yes	Yes	No

3.5 DISCUSSION

We aimed to characterise the human reward system in a large sample of adolescents. Given that previous studies of reward processing have used nominally small sample sizes it has previously been hard to determine the consistency and robustness of available random effect studies. Here we show random effects analysis of reward anticipation and reward feedback in a large sample of adolescents, which gives us greater statistical power to determine even small changes in brain activation during reward processing.

3.5.1 RANDOM EFFECTS ANALYSES OF REWARD ANTICIPATION AND FEEDBACK

The analyses performed here were based on two contrasts of the MID task: 'reward anticipation high win vs. reward anticipation no win' and 'reward feedback high win vs. reward feedback no win'. By investigating activation patterns during the 'high win vs. no win' contrasts, rather than the 'high win vs. low win' contrasts, we were able to capture as much of the signal associated with reward processing as possible. We chose the 'high win vs. no win' contrasts, as opposed to 'high win vs. baseline', in order to minimise the variance related to non-reward processes, such as visual processing.

As expected based on research of the adult reward system, the random effects analysis of the 'anticipation high win vs. no win' contrast revealed BOLD-responses extending from the striatum. The cluster was very large ($k > 47{,}465$ voxels) and extended to prefrontal and middle frontal cortex as well as to the parietal and occipital lobes. During the 'feedback high win vs. no win' contrast, BOLD-responses were restricted to the anterior and posterior cingulate gyrus, medial OFC and parietal lobe. There was little overlap in the activation patterns from the two contrasts. The overlap was centered in the cingulate gyrus and superior parietal lobule.

Prior research of the adult reward system suggests that the VS is a key activated region in both phases of reward processing (Knutson, Fong, et al., 2001; Liu, et al., 2011). However, we did not find activation of this region during the 'reward high win feedback vs. reward no win feedback' contrast. Results by Schultz and colleagues suggested that before learning the association between a cue and a reward, dopaminergic neurons in the midbrain will fire after the reward has been presented (Schultz, et al., 1997). However, once the association has been learned, dopaminergic neurons will fire in response to the cue. Considering that our participants had learned the association between the cue and the reward in the high win trials, this may explain why we see very little activation of the VS during the reward feedback phase. Another explanation for the discrepancy between our results and results presented by Liu and colleagues may be that our findings are based on adolescents, which have been suggested to show reduced VS activation during reward processing relative to adults (Bjork, et al., 2004). It is possible that adults would still show the significant activation of the VS during both phases of reward processing.

We also investigated whether the OFC is activated during one or both stages of the MID task. During the reward anticipation phase the activation in the OFC is in the middle section, while during reward feedback the activations shift to the medial OFC. The medial OFC is related to the monitoring, learning and memory of the reward value of reinforcers, whereas the middle OFC may play a role in response inhibition and the evaluation of losses (Kringelbach, 2005; Sescousse, et al., 2010). During reward anticipation our participants had to withhold their responses while waiting for the target (i.e. the white square) to appear. This may explain the activation in the OFC during the anticipatory stages of the MID. During reward feedback our participants were informed of the reward received in a particular trial and overall

winnings. This may have given them the opportunity to monitor and learn how their responses had affected the outcome.

Findings from Liu and colleagues' meta-analysis of reward processing suggest that a distributed network of regions are involved in reward anticipation, including the striatum, but also the cingulate cortex, middle frontal gyrus, parietal lobule and premotor regions. During reward feedback, the meta-analysis suggested activation of the striatum, cingulate gyrus, supplementary motor area and prefrontal cortex (see **Supplementary Table 1)**. Thus, the results from this study of adolescents are largely in agreement with results of the meta-analysis, with the exception of striatal activation during reward feedback. This discrepancy between our findings and those of the meta-analysis may be the result of a younger cohort investigated in the IMAGEN study. It is also possible that activation during reward feedback is particularly task-dependent. The meta-analysis included reward studies of many different types of reward-tasks, including tasks of reward decision making.

3.5.2 Overlap between reward anticipation and feedback

The results from this study suggest that there is overlap between brain activations during reward anticipation and reward feedback in the superior parietal lobule, cingulate gyrus and hippocampus. The superior parietal lobule was activated during reward anticipation, but some activation was also seen during reward feedback. Similar to the OFC, the parietal lobule has been associated with the valuation of different options and the integration of information. The cingulate gyrus has previously been implicated in interoception and empathy as well as risk and uncertainty assessment lending its role in reward anticipation (Craig, 2002; Gu et al., 2010). It is also suggested to relay information from subcortical regions to the frontal cortex which may explain why the same region is activated during both contrasts. It is

interesting to note that the hippocampus is activated during both stages of reward processing. It was recently suggested that the hippocampus codes for uncertainty of the association between reward-related cues and reward feedback (Vanni-Mercier, Mauguiere, Isnard, & Dreher, 2009).

3.5.3 LIMITATIONS

Our study has a couple of methodological limitations. Firstly, the current study only tested brain responses to rewards, not punishment, and only manipulated the magnitude of reward (high win, small win, no win). Other factors known to affect reward processing (probability, expected value, timing, uncertainty) were not tested. Secondly, the current study investigated reward processing in 13-15 year old adolescents. Considering that we did not test reward processing in adults, we are unable to determine whether the adult reward system is activated in a similar manner.

3.5.4 CONCLUSIONS

Functional MRI enabled us to characterise widespread activation, with a peak in the VS, during reward anticipation and activation of the cingulate gyrus and medial OFC during the reward feedback phase. Our findings are largely consistent with the prior meta-analysis of the reward processing in humans, but we also extend these findings in a data-driven manner to identify brain regions beyond those denoted in previous literature.

4 CHAPTER FOUR:

GENDER DIFFERENCES IN REWARD PROCESSING AND ADHD SYMPTOMS

4.1 OBJECTIVES OF THIS CHAPTER

In this chapter gender differences in brain activation during reward anticipation and reward feedback are explored. The relationship between ventral striatal (VS) activation during reward processing and symptoms of Attention Deficit Hyperactivity Disorder (ADHD) were explored amongst boys and girls separately. The specific objectives of this chapter are as follows:

1. Determine whether there are gender differences in VS activation during reward anticipation
2. Determine whether there are gender differences in ventral striatal activation during reward feedback
3. Explore whole brain gender differences in reward anticipation
4. Explore whole brain gender differences in reward feedback
5. Replicate the negative correlation between VS activation during reward processing and ADHD symptoms reported in the literature
6. Investigate whether there are gender differences in the correlation between VS activation during reward processing and ADHD symptoms

4.2 INTRODUCTION

Gender differences are frequently reported in reward sensitivity and reward dependence (Corr, 2004, 2008; C. s R. Li, Huang, Lin, & Sun, 2007; Lucas, Diener, Grob, Suh, & Shao, 2000; Torrubia, Avila, Molto, & Caseras, 2001). Furthermore, gender differences are frequently reported in externalising disorders known to be associated with reward sensitivity and aberrant reward processing (Arnold, 1996; Hasson & Fine, 2012; Luman, van Meel, Oosterlaan, & Geurts, 2012; Tripp & Alsop, 2001). However, the neurobiology mediating gender differences in personality and disorders is not well understood. This chapter investigates gender differences in reward processing measured by the MID task in a large sample of adolescents. Considering that many studies of reward processing in ADHD focus exclusively on males (Paloyelis, Mehta, Faraone, Asherson, & Kuntsi, 2012; Strohle, et al., 2008), we also explored whether there are gender differences in the relationship between ADHD symptoms and VS activation patterns.

On the basis of personality questionnaire data, gender differences have been reported in sensitivity to reward and reward dependence. In the Cloninger's United States normative data, women scored higher than men on reward dependence (Cloninger, et al., 1991). These findings supported previous work by (Nixon & Parsons, 1989). Other personality questionnaire studies, using the sensitivity to punishment and sensitivity to reward questionnaire (SPSRQ), suggest that men score significantly higher on the scale of reward sensitivity relative to females. This finding was first presented by Torrubia and colleagues and later replicated in a large sample of college students (C. s R. Li, et al., 2007; Torrubia, et al., 2001). These early studies were unable to determine neurobiological reasons underlying gender differences in reward sensitivity or reward dependence.

Whereas reward sensitivity and reward dependence have been investigated using personality questionnaire data for many decades, the neurobiology of reward processing has only been investigated using functional MRI measures since the beginning of the century. The MID task is the most frequently used measure of reward processing in functional MRI studies (Knutson, et al., 2000; Knutson, Fong, et al., 2001), as it measures brain activation during both reward anticipation and reward feedback.

Few studies have explicitly investigated gender differences in the neurobiology of reward processing. Spreckelmeyer and colleagues explored gender differences in neural responses to two common forms of human reward: money and social approval (a smiling face) (Spreckelmeyer, et al., 2009). In response to increasing levels of monetary rewards, men showed stronger activation in the left putamen relative to women. In response to increasing levels of social rewards, women showed stronger activation than men in the left caudate. Men also displayed a wider network of brain areas sensitive to the increasing level of monetary reward compared to women, contrasted by only little activation in response to increasing levels of social rewards. Women, on the other hand, displayed equal cortical activation patterns with respect to increasing levels of monetary and social rewards. These data suggest that there are gender differences in reward-related brain activation in adults. However gender differences in reward processing during adolescence have not been investigated (Galvan, et al., 2006).

Functional MRI studies of reward processing have not previously identified gender differences in VS activation, which is believed to be the key region underlying reward processing in humans (Knutson, Fong, et al., 2001; Liu, et al., 2011; Schott, et al., 2008; Schultz, et al., 1997). However, positron emission tomography (PET)

studies of humans suggest that dopaminergic affinity within this region differs by gender (Munro, et al., 2006; Pohjalainen, Rinne, Nagren, Syvalahti, & Hietala, 1998).

Skewed gender ratios in many reward-related disorders are another reason for investigating gender differences in reward processing (Arnold, 1996). Externalising disorders, such as ADHD, are frequently associated with aberrant reward processing (Carmona, et al., 2011; Scheres, Milham, Knutson, & Castellanos, 2007b; Strohle, et al., 2008). Evidence from neuroimaging studies indicates that ADHD patients show reduced activation of the VS during reward anticipation relative to healthy controls. Some of these studies also investigate the correlations between symptom-counts, rather than diagnosis, and VS activation. A couple of studies show that self-rated and parent-rated ADHD symptoms are negatively correlated with VS activation in ADHD patients (Scheres, et al., 2007b; Strohle, et al., 2008). However, to date, the negative correlation between VS activation and symptom count has not been identified in healthy participants.

Many neuroimaging studies of ADHD have been performed in males only (Paloyelis, et al., 2012; Scheres, et al., 2007a; Stoy et al., 2011; Strohle, et al., 2008). This is a partly a reflection of the skewed gender ratios in ADHD, but many studies also aim to recruit a homogeneous sample in order to reduce the number of covariates in their analyses. Although some studies have recruited both male and female participants, gender differences in reward-related brain activation of ADHD patients have not been investigated (Carmona, et al., 2011). This is possibly due to small sample sizes, which lack sufficient power to investigate associations between VS activation and ADHD by gender. We wanted to determine whether the association between ADHD and VS activation is specific to males.

In sum, gender differences in reward processing are not well understood and little is known about gender differences in reward processing in ADHD. We explored gender differences in BOLD-responses during reward processing in a large sample of adolescents (n = 1,234). Knowing that reward processing is frequently associated with ADHD, we also investigated potential gender differences in the association between VS BOLD-responses and ADHD symptoms.

4.3 MATERIAL AND METHODS

4.3.1 PARTICIPANTS

We used data from the full sample of IMAGEN (n = 2,030). Individuals who had complete data of the anticipation and outcome phase of the MID task (n = 1,860), passed task specific outlier criteria, particularly in terms of movement (n = 1,623), passed contrast-specific outlier criteria in terms of spike detection control (n = 1,384), had been able to see the task in the scanner (n = 1,374), had complete handedness data (n = 1,364), had complete IQ data (> 75) (n = 1,256), did not show structural abnormalities (n = 1,243) and had complete data on the Strength and Difficulties Questionnaire (n = 1234). This left 1,234 adolescents passing all criteria (n = 579 boys, n = 655 girls). The sample had a mean age of 14.4 years (SD: 0.4; range: 13.2-15.7 years) (see ***Table 6*** for demographics). Participants were tested in eight IMAGEN assessment centres (London, Nottingham, Dublin, Mannheim, Berlin, Hamburg, Paris & Dresden). The study was approved by local ethics research committees at each site. A detailed description of recruitment and assessment procedures, as well as in/exclusion criteria can be found elsewhere (Schumann, et al., 2010). One thousand and eighty two participants were right-handed and 152 participants were left-handed or ambidextrous. Individuals with verbal or

performance IQ < 75 were removed from further analysis. Handedness and study site were controlled for in all analyses.

Table 6. Demographics for total sample, split by gender: means, standard deviations and ranges are shown below (Mean ± SD, (*Range)).*

	ADHD symptoms	***Age***	***PIQ***	***VIQ***
Total (n = 1,234)	2.8 ± 2.2 *(0-10)*	14.4 ± 0.4 *(13.3-15.7)*	108.5 ± 13.7 *(76-147)*	111.4 ± 14.6 *(76-155)*
Boys (n = 579)	3.2 ± 2.3 *(0-10)*	14.4 ± 0.4 *(13.3-15.5)*	108.5 ± 14.2 *(76-147)*	113.8 ± 14.9 *(76-155)*
Girls (n = 655)	2.4 ± 2.1 *(0-10)*	14.4 ± 0.4 *(13.3-15.7)*	108.6 ± 13.2 *(76-147)*	110.8 ± 14.3 *(76-152)*

ADHD symptoms: Impulsivity, Hyperactivity and Inattentiveness measured by SDQ; PIQ: Performance IQ, VIQ: Verbal IQ

4.3.2 MONETARY INCENTIVE DELAY (MID) TASK

The participants performed a modified version of the MID task to study neural responses to reward anticipation and reward feedback. The paradigm has been described in a previous publications (Nees, et al., 2012)(or see **Section 2.4.1**).

4.3.3 FMRI DATA ACQUISITION AND ANALYSIS

Structural and functional MRI data were acquired at eight IMAGEN assessment sites with 3T MRI scanners of different manufacturers as described in **Section 2.4.3**. The second level whole brain analysis of gender effects in reward anticipation and reward feedback (design: two-sample t-test) included handedness (right/ambidextrous) and 7 dummy-coded centre covariates (see ***Figure 9*** and ***Figure 10*** for second-level models). The VS ROI was extracted based on previous research (Yacubian, et al.,

2006) (xyz = ±15, 9, -9, radius of 9 mm) using Marsbar (http://marsbar.sourceforge.net).

4.3.4 ADHD SYMPTOMS

ADHD symptoms were assessed using parental reports of the Strengths and Difficulties Questionnaire (SDQ), a brief 25-item behavioural screening tool probing for ADHD type problems (hyperactivity, inattention and impulsivity), emotional symptoms, conduct problems, peer problems and prosocial behaviour (see **Section 2.3.3** and **Appendix 1**). The current study used parental reports on ADHD symptoms, as externalising problems in children have been shown to be more reliably reported by parents than by self-report (Herjanic & Reich, 1997). No participant was identified as a 'probable' case of ADHD according to SDQ likelihood ratings.

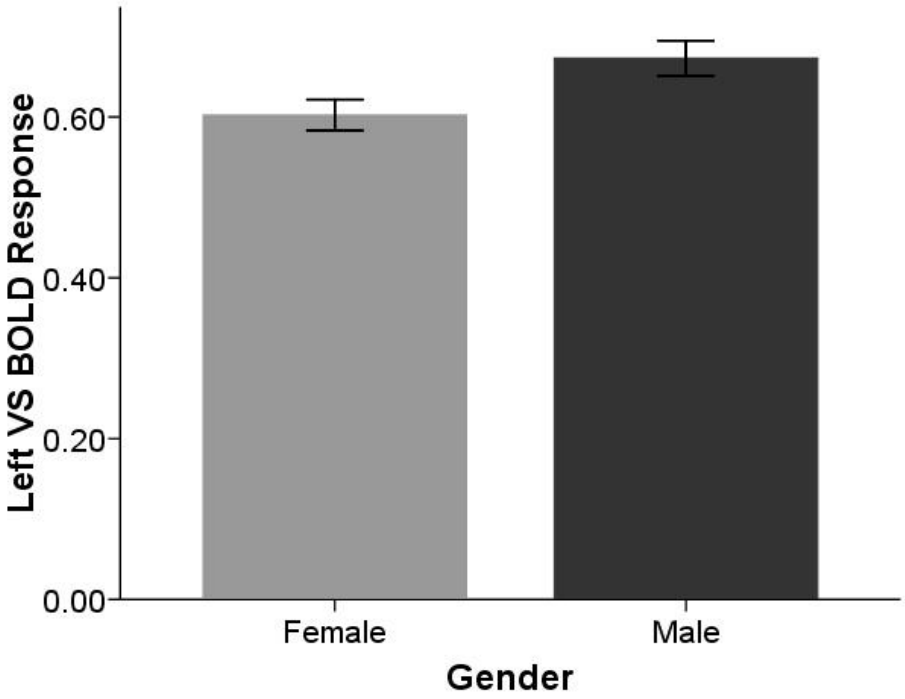
4.4 RESULTS

4.4.1 REGION OF INTEREST ANALYSIS OF THE VENTRAL STRIATUM

4.4.1.1 Gender Difference During Reward Anticipation

Using ROI analyses, we found gender differences in left VS activation during reward anticipation (t = 7.17, p = 0.008, partial eta squared = 0.006) and a trend in right VS activation (t = 3.52, p = 0.061). Boys showed significantly higher activation of the bilateral VS compared to girls (***Figure 6***).

Figure 6. Gender differences in left VS activation during reward anticipation, suggesting significantly higher activation of the left VS in boys relative to girls (t = 7.17, p = 0.008, partial eta squared = 0.006).



4.4.1.2 *Gender Differences During Reward Feedback*

We found significant gender differences in the left VS (t = 10.01, p = 0.002, partial eta squared = 0.008) and right VS (t = 12.17, p = 0.001, partial eta squared = 0.01) during reward feedback. Again, boys showed significantly higher activation of the VS compared to girls (***Figure 7*** and ***Figure 8***).

Figure 7. Gender differences in left VS activation during reward feedback, suggesting significantly higher activation of the left VS in boys relative to girls (t = 10.01, p = 0.002, partial eta squared = 0.008).

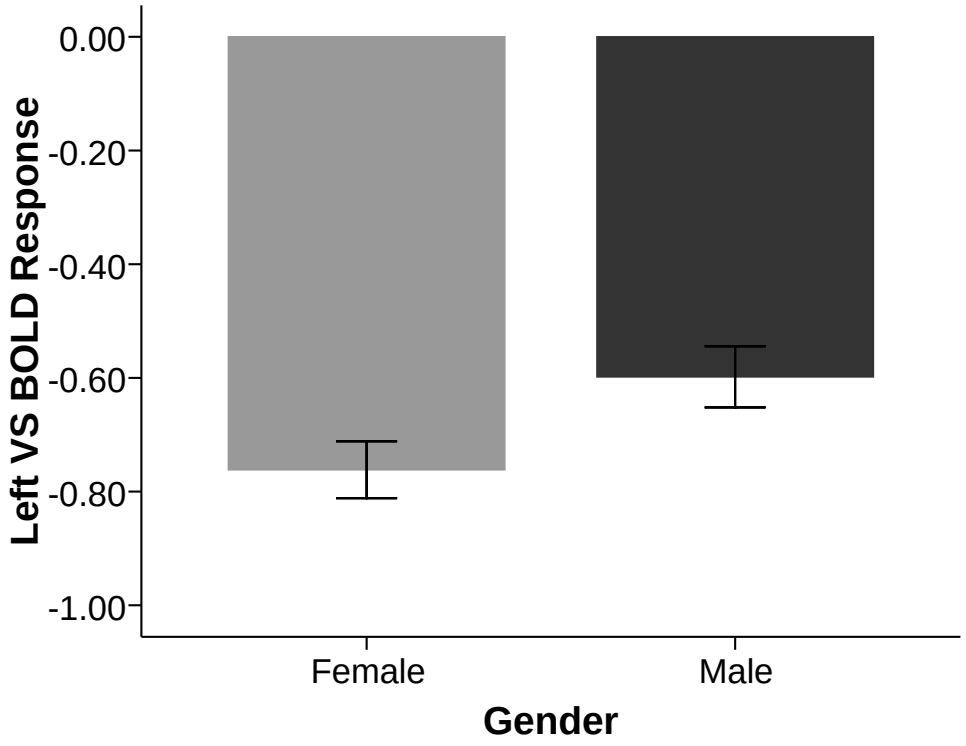
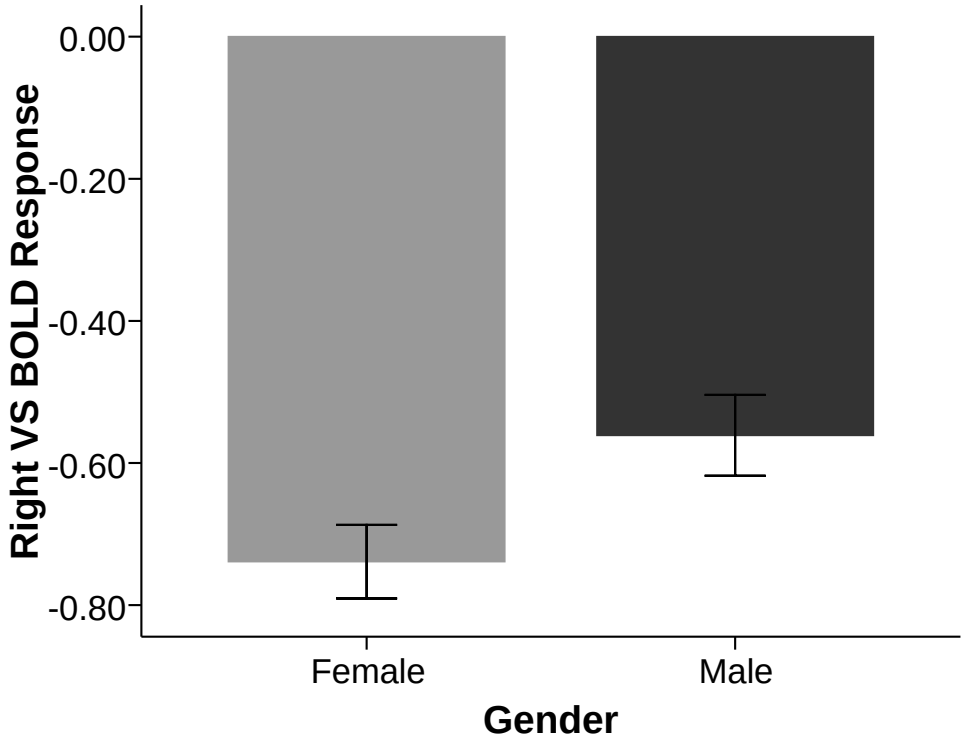


Figure 8. Gender differences in right VS activation during reward feedback suggested significantly higher activation of the right VS in boys relative to girls (t = 12.17, p = 0.001, partial eta squared = 0.01).



4.4.2 WHOLE BRAIN ANALYSES

4.4.2.1 Gender Differences During Reward Anticipation

A whole brain t-test identified significant gender differences during reward anticipation. All gender differences suggest that boys show higher BOLD-responses compared to girls. Regions where gender differences were displayed, along with p-values, Z-scores and cluster sizes, are summarised in ***Table 7***. Relative to girls, boys showed significantly higher activation of subcortical regions including the caudate (x,y,z: 12, 11, 7; Z = 5.34; $p_{FWE\text{-}corrected}$ = 0.001) and cingulate gyrus (x,y,z: 9, 11, 34; Z = 4.26; $p_{FWE\text{-}corrected}$ = 0.004), frontal regions including the bilateral superior frontal gyrus (right: x,y,z: 30, 26, 55; Z = 5.38; $p_{FWE\text{-}corrected}$ < 0.001; left: x,y,z: -36, 20, 49; Z = 5.06; $p_{FWE\text{-}corrected}$ < 0.0001) as well as the precentral (x,y,z: -36, -4, 37; Z = 3.80;

$p_{FWE\text{-}corrected}$ = 0.016) and postcentral gyrus (x,y,z: -45, -22, 40; Z = 4.96; $p_{FWE\text{-}corrected}$ < 0.0001). The whole brain analysis also revealed that boys show significantly higher BOLD-responses in the superior temporal gyrus (x,y,z: -48, -22, 1; Z = 5.68; $p_{FWE\text{-}corrected}$ < 0.0001), inferior temporal gyrus (x,y,z: 57 -55 -11; Z = 5.23; $p_{FWE\text{-}corrected}$ < 0.0001) and precuneus (x,y,z: 6, -70, 40; Z = 5.82; $p_{FWE\text{-}corrected}$ < 0.0001) (***Figure 9***). The inverse comparison of girls > boys did not yield any significant clusters.

Figure 9. Second level model of anticipation large win vs. no win to determine gender differences (boys > girls) and the resulting whole brain activation patterns associated with higher activation in boys relative to girls ($p_{FWE\text{-}corrected}$ < 0.05).

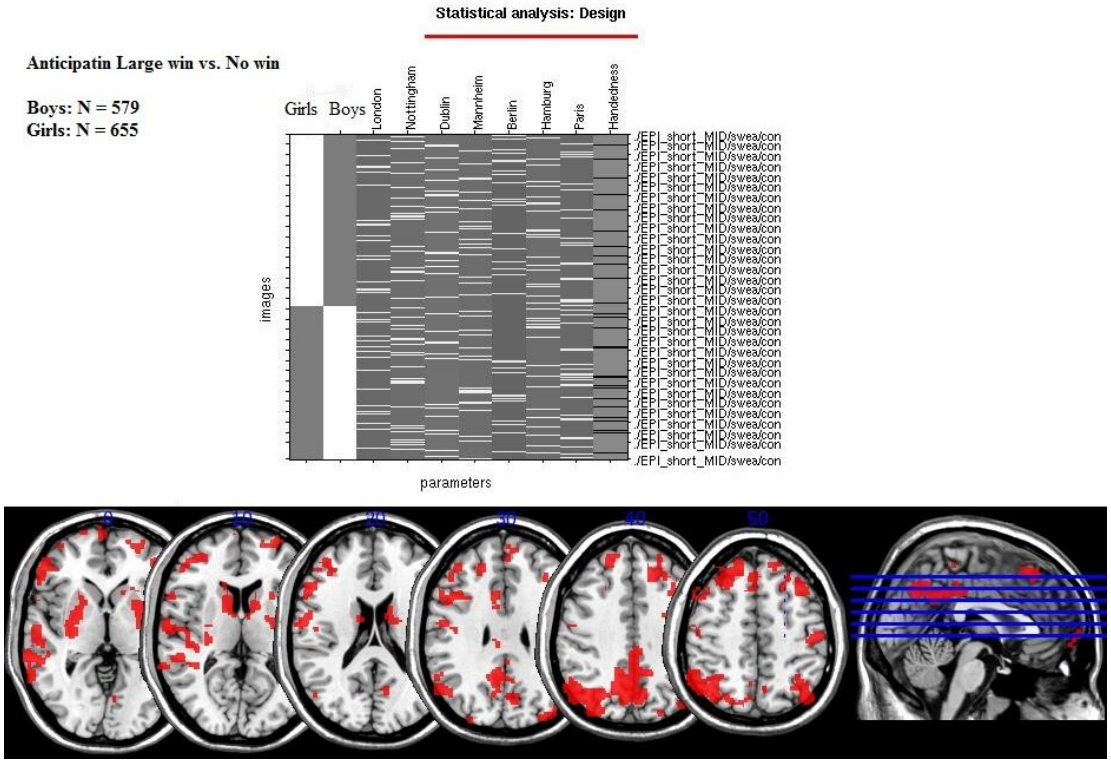


Table 7. Gender differences in activation patterns during reward anticipation (boys > girls)

Brain area	***MNI coordinates***	***Z-score***	***Cluster size (k)***	$P_{FWE\text{-}corrected}$
L Postcentral Gyrus	-15 -34 67	5.29	120	<0.0001
L Postcentral Gyrus	-45 -22 40	4.96	203	<0.0001
L Precentral Gyrus	-36 -4 37	3.8	50	0.016
L Precuneus	-39 -79 37	4.35	257	<0.0001
L Superior Frontal Gyrus	-36 20 49	5.06	369	<0.0001
L Superior Temporal Gyrus	-48 -22 1	5.68	1794	<0.0001
R Caudate	12 11 7	5.34	79	0.001
R Cingulate Gyrus	9 11 34	4.26	64	0.004
R Inferior Temporal Gyrus	57 -55 -11	5.23	479	<0.0001
R Lentiform Nucleus	27 2 -8	5.33	475	<0.0001
R Precuneus	6 -70 40	5.82	1241	<0.0001
R Superior Frontal Gyrus	3 68 1	4.38	39	0.046
R Superior Frontal Gyrus	30 26 55	5.38	1071	<0.0001

4.4.2.2 *Gender Differences During Reward Feedback*

We found significant whole-brain differences between how boys and girls process reward feedback, with boys showing significantly higher BOLD-responses compared to girls in the caudate (x,y,z = 18, 5, 22; Z = 6.04; $p_{FWE\text{-}corrected}$ = 0.015) and cerebellum (x,y,z = 57 -55 -26, Z = 6.14; $p_{FWE\text{-}corrected} < 0.0001$) (***Table 8***, ***Figure 10***). The inverse comparison of girls > boys did not yield any significant clusters.

Figure 10. Second level model of feedback large win vs. no win to determine gender differences (boys > girls) and the resulting whole brain activation patterns associated with higher activation in boys relative to girls ($p_{FWE\text{-}corrected} < 0.05$).

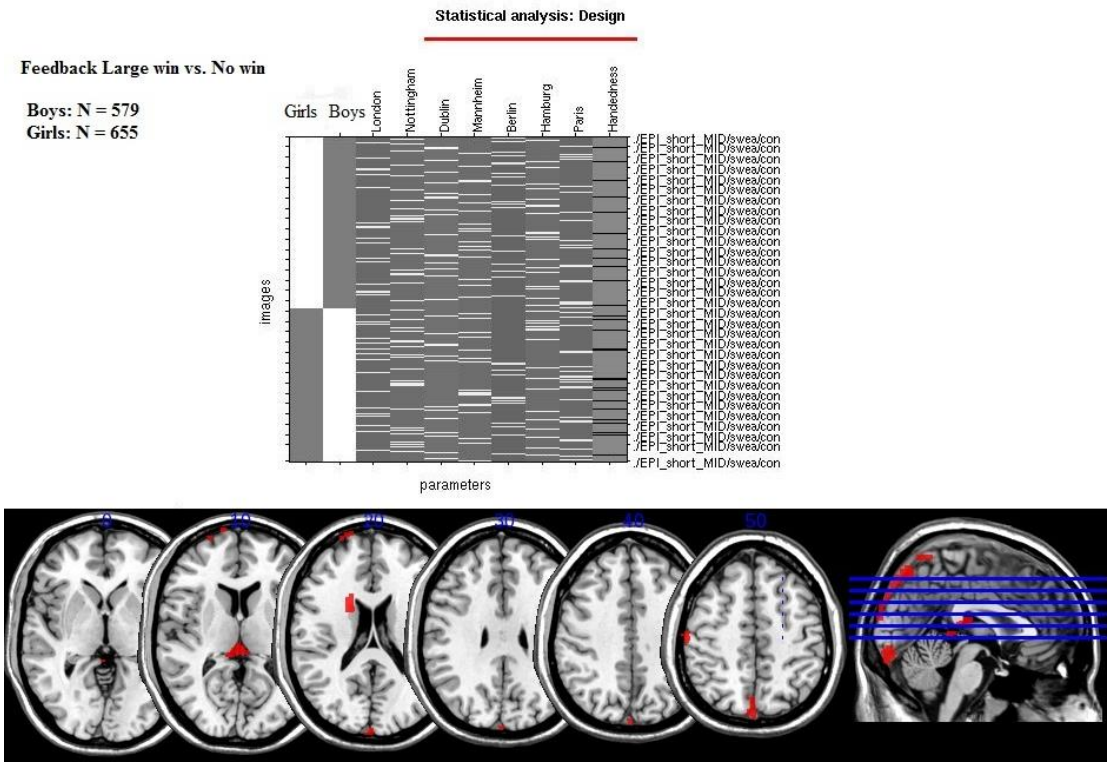


Table 8. Gender differences in activation patterns during reward feedback (boys > girls)

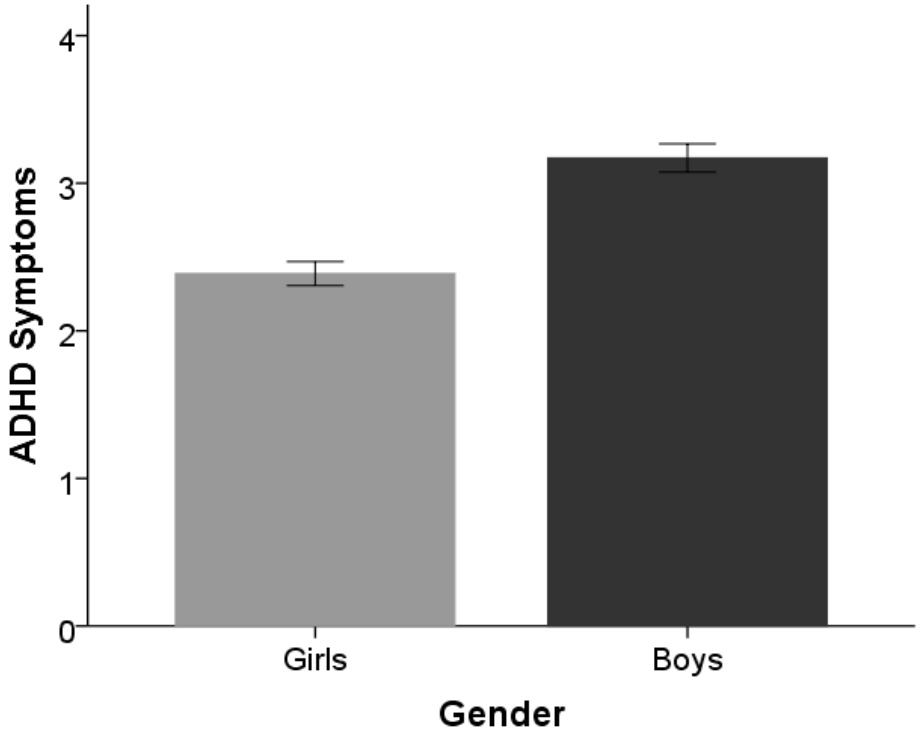
Brain area	***MNI coordinates***	***Z-score***	***Cluster size (k)***	$P_{FWE\text{-}corrected}$
R Caudate	18 5 22	6.04	49	0.015
R Cerebellum	57 -55 -26	6.14	189	<0.0001

4.4.3 ADHD SYMPTOMS

4.4.3.1 Gender Differences in ADHD Symptoms

We found significant gender differences in ADHD symptoms (t = 38.59, p < 0.0001, partial eta squared = 0.03), with boys (mean score: 3.17) scoring significantly higher than girls (mean score: 2.39) (see ***Figure 11***).

Figure 11. ADHD symptoms in boys and girls: Boys show significantly higher levels of ADHD symptoms relative to girls (t = 38.59, p < 0.0001, partial eta squared = 0.03).



4.4.3.2 Correlations between ADHD Symptoms and VS During Reward Anticipation

A significant negative correlation was identified between ADHD symptoms and left VS activation (r = -0.068, p = 0.017) as well as right VS activation (r = -0.074, p = 0.009) in the full sample. When dividing the sample by gender we found a negative correlations between ADHD symptoms and left VS activation (r = -0.094, p = 0.024) and right VS activation (r = -0.111, p = 0.008) in boys only. Again, the effect sizes

were small. No significant correlations were identified for girls (left VS: r = -0.068, p = 0.082; right VS: r = -0.053, p = 0.18).

4.4.3.3 Correlations between ADHD Symptoms and VS During Reward Feedback

In the full sample, significant positive correlations were identified between ADHD symptoms and left VS activation (r = 0.076, p = 0.008) as well as between ADHD symptoms and right VS activation (r = 0.074, p = 0.010). When the sample was split by gender, we identified a trend towards a positive correlations in the left VS (r = 0.075, p = 0.074) and in the right VS (r = 0.074, p = 0.075) amongst boys. No significant correlations were identified for the girls (left VS: r = 0.054, p = 0.167; right VS: r = 0.044, p = 0.267).

4.4.3.4 Correlations between ADHD Symptoms and the Differential VS Activation between Reward Anticipation and Reward Feedback

In order to determine whether ADHD symptoms are associated with the relationship between VS activation during reward anticipation and reward feedback we subtracted VS activation during reward feedback from VS activation during reward anticipation ($VS_{Anticipation} - VS_{Feedback}$). We found that $VS_{Anticipation} - VS_{Feedback}$ was significantly correlated with ADHD symptoms in the full sample (left VS: r = -0.075, p = 0.009; right VS: r = -0.072, p = 0.011). When the sample was split by gender, we found a significant negative correlation between ADHD symptoms and $VS_{Anticipation} - VS_{Feedback}$ in boys (left VS: r = -0.088, p = 0.035; right VS: r = -0.094, p = 0.024), suggesting that less sensitivity to the reward cue is associated with greater ADHD symptoms. The correlation between ADHD symptoms and $VS_{Anticipation} - VS_{Feedback}$ was not significant in girls (left VS: r = -0.064, p = 0.102; right VS: r = -0.051, p = 0.195).

4.5 DISCUSSION

This chapter investigated gender differences in reward processing using a large sample of adolescents. We also explored gender differences in the association between ADHD symptoms and VS activation.

4.5.1 GENDER DIFFERENCES IN VS ACTIVATION

Gender differences have been reported in questionnaire-data of reward sensitivity and reward dependence. However, imaging studies of reward processing rarely investigate gender differences in neural function. This may be due to the small sample sizes of neuroimaging datasets, which do not have the power to investigate gender effects. Studies may also choose to recruit only boys or only girls in order to keep the sample homogeneous. The results of this study suggest that neural responses to reward anticipation and reward feedback differ by gender in adolescents. Boys show significantly higher activation of the VS during both reward anticipation and reward feedback. Gender differences in reward processing may provide a reason to why males and females differ in reward sensitivity and reward dependence and may also provide a reason to why males and females differ in their vulnerability to a number of reward-related disorders. However, it should be noted that although t-tests of gender differences in VS activation were highly significant, the effect sizes were small (partial eta squared = 0.006-0.01).

Studies suggest that dopamine release in the brain and the VS in particular, differ between boys and girls. It has been shown that boys have markedly increased dopaminergic affinity in the brain compared to girls (Munro, et al., 2006; Pohjalainen, et al., 1998). Gender differences observed in VS activation during reward anticipation and reward feedback may reflect the increased dopaminergic affinity in boys relative to girls. PET studies suggest that gender differences in

dopaminergic affinity are particularly pronounced in puberty (Kuhn et al., 2010; Munro, et al., 2006; Pohjalainen, et al., 1998). Unfortunately, in this study boys are at an earlier stage of pubertal development relative to girls, which precludes investigations of gender differences in VS activation independent of pubertal development.

4.5.2 GENDER DIFFERENCES IN WHOLE-BRAIN ANALYSES

Many neuroimaging studies of reward processing focus solely on VS activation. However, the results of Chapter Three suggest that reward anticipation and reward feedback activates a large network of regions. The results of this chapter show that gender differences in reward processing are not confined to the VS. Boys showed significantly higher activation of the caudate, prefrontal cortex and premotor cortex relative to girls during reward anticipation. As part of the striatum, the caudate is a receiver of dopaminergic neurons from the VTA (Knutson, Adams, et al., 2001). As discussed above, higher activation of the striatum may reflect higher dopaminergic affinity within this region amongst boys. The striatum is connected with the OFC through the mesocortical pathway. The main function of the frontal cortex during reward processing is to make value guided decisions about future behaviour. Boys also show significantly higher activation of the premotor cortex compared to girls, which may reflect a higher level of motoric preparedness to increasing reward amongst boys. The fact that gender differences are found in BOLD-responses of known reward processing regions suggests the importance of controlling for the effect of gender in both region of interest analyses and whole brain analyses. These results also highlight the importance of investigating reward-related activation patterns in boys and girls separately.

During reward feedback, we found significant gender differences in the caudate and cerebellum. Again, boys showed higher activation of these regions relative to girls. The cerebellum is frequently activated during reward processing (Knutson, Fong, et al., 2001; Liu, et al., 2011; Thoma, Bellebaum, Koch, Schwarz, & Daum, 2008) and has recently been discussed in terms of involvement in reward-based associative learning. Precise event-timing, such as pressing a button, might be one of the critical components coordinated by the cerebellum during reward-based learning (Ivry, Spencer, Zelaznik, & Diedrichsen, 2002; Thoma, et al., 2008).

4.5.3 GENDER DIFFERENCES IN RELATIONSHIP BETWEEN ADHD SYMPTOMS AND VS ACTIVATION

In the full sample of boys and girls, VS BOLD-responses were significantly correlated with ADHD symptoms. The effect sizes of these results were very small (reward anticipation: $r \sim -0.07$; reward feedback: $r \sim 0.07$); however, it is interesting to note that our results are consistent with the ADHD literature which suggests reduced activation of VS in ADHD patients compared to healthy controls as well as significant negative correlations between VS activation and ADHD symptoms in ADHD patients (Scheres, et al., 2007b; Strohle, et al., 2008). The fact that our results were based on a community sample of healthy adolescents may explain the low effect sizes. Previous research has reported significant associations between ADHD status and activation of the OFC during reward feedback. However, the relationship between ADHD symptoms and VS activation during reward feedback has not previously been reported.

As predicted, the negative correlation between VS activation during reward anticipation was driven by boys. This suggests that the frequently reported negative association between VS activation and ADHD diagnosis may be gender-specific.

Future research may want to replicate these findings in a clinical population of boys and girls to determine whether larger effect sizes can be identified. The relationship between ADHD symptoms and VS activation during reward feedback was not significant when analysed in boys and girls separately.

Considering that boys, who show significantly higher VS activation relative to girls, also show a higher level of ADHD symptoms, it is curious that we observed a significant negative correlation between ADHD symptoms and VS activation. The fact that girls show significantly lower VS activation and lower levels of ADHD symptoms may lead us to infer that reduced VS activation is protective against ADHD. However, in boys, ADHD symptom-count appeared to increase as VS activation is reduced. We may postulate that boys have a different baseline of VS activation relative to girls which makes them more prone to develop ADHD symptoms if their VS activation patterns are not sufficiently high. However, theories of dopaminergic dysfunction in ADHD suggest that it is not VS activation during anticipation as such that is associated with ADHD, but it is rather the relationship between the phasic and tonic dopaminergic patterns during reward anticipation *and* reward feedback that underlie ADHD symptoms as proposed by (Tripp & Wickens, 2008) in the dopamine transfer deficit (DTD) theory. The DTD proposes that in children with ADHD the phasic dopamine cell response to the 'cue' that predicts reinforcement is reduced in amplitude to the point of being ineffective, although the timing of this cue is normal. In the absence of an anticipatory dopamine signal even short delays are likely to influence the effectiveness of reinforcement. This notion is supported by the negative correlation between ADHD symptoms and $VS_{Anticipation} - VS_{Feedback}$. However, it should be noted that these findings are in need of replication in a clinical population.

4.5.4 LIMITATIONS

A few limitations to our study should be noted. Firstly, this study was limited by the significantly lower level of ADHD symptoms amongst girls relative to boys. The low ADHD symptom-count in girls may have been insufficient to explicitly test the relationship between VS activation and ADHD symptoms. The results need to be replicated in a clinically diagnosed cohort of boys and girls.

Secondly, this study targeted a narrow age-span of 13-15 year old adolescents, during which puberty development varies by gender. According to the puberty development scale (PDS) all boys in our sample scored within the range 'prepubescent' to 'mid-pubescent' whereas all girls scored within the range 'mid-pubescent' to 'post-pubescent'. Thus, we were unable to determine whether reward-related BOLD-responses differ by gender, independently of pubertal development.

Thirdly, striatal dopamine receptor binding of D_1 and D_2 receptors have been shown to peak in adolescence at levels that are about 30-45% greater than those seen in adulthood (Tarazi, Tomasini, & Baldessarini, 1999; Teicher, Andersen, & Hostetter, 1995). Thus, it is unclear whether these findings would generalise to an adult population. Longitudinal MRI-studies of the developing reward system are necessary in order to determine gender differences across ages and pubertal stages.

Finally, we used a community based cohort which does not allow investigation of ADHD patients vs. healthy controls. No participant within our dataset was labeled as a probable case of ADHD according to the SDQ. Thus, we were unable to determine whether gender differences in reward processing mediate the disorder of ADHD.

4.5.5 CONCLUSIONS

The MID task is one of the most frequently used functional MRI measures of reward-related processing in the literature. To our knowledge this is the first report to suggest significant gender differences in reward processing in adolescents. Our findings show the importance of separately investigating the relationship between behaviour and BOLD-responses during reward processing in boys and girls. If this is not possible due to small sample sizes we suggest controlling for gender effects when performing neuroimaging studies of the reward system.

5 CHAPTER FIVE:

MAOA GENOTYPE AFFECTS VENTRAL STRIATAL ACTIVATION IN BOYS, BUT NOT IN GIRLS

5.1 OBJECTIVES OF THIS CHAPTER

The objective of this chapter is to identify whether ventral striatal (VS) activation is associated with novelty seeking and whether this relationship differs by gender. We also explored whether the X-linked gene Monoamine Oxidase A *(MAOA)* is associated with VS activation in boys, girls or both genders and whether correlations between VS activation and novelty seeking differ by *MAOA* genotype. The specific aims of this chapter are as follows:

1. Investigate whether VS activation is associated with Temperament and Character Inventory (TCI) novelty seeking scores
2. Explore whether the relationship between VS activation and novelty seeking differs by gender
3. Determine whether *MAOA* is associated with the measure of novelty seeking provided by the TCI
4. Determine whether *MAOA* genotype is associated with VS activation during reward anticipation, and whether the effect of *MAOA* differs by gender
5. Explore whether the relationship between VS activation and novelty seeking differs by *MAOA* genotype

5.2 INTRODUCTION

Adolescents who show high levels of novelty seeking are likely to pursue exciting, but potentially dangerous, activities (Wills, Vaccaro, & McNamara, 1994; M. Zuckerman & Kuhlman, 2000). Novelty seeking in adolescence is a predictor of smoking, alcohol use, drug use and other risky behaviours (Peters et al., 2011; Schneider et al., 2012). It is suggested that adolescents experience novelty seeking to be rewarding. Neuroimaging studies have investigated the potential link between novelty seeking and reward processing in adolescence. Functional MRI studies indicate that novelty seeking behaviours during adolescence stem from immaturities in the brain circuitry mediating reward processing (C. Geier & Luna, 2009; C. F. Geier, et al., 2010; Telzer, et al.).

Several studies suggest that activation of the VS during reward processing is associated with novelty seeking in adolescence. Some studies suggest a positive correlation between ventral striatal activation during reward processing and risky behaviours across development (Galvan, et al., 2007; Krebs, Schott, & Duzel, 2009). Other studies suggest that with increasing risk-taking bias, the VS show decreased activation during reward anticipation (Peters, et al., 2011; Schneider, et al., 2012). Thus, a discrepancy exists regarding whether overactivation or underactivation of the VS results in novelty seeking in adolescence.

Research also suggest gender differences in both novelty seeking and reward sensitivity, with males showing a greater level of novelty seeking and a lower level of reward sensitivity relative to females (C. s R. Li, et al., 2007; Torrubia, et al., 2001; M. Zuckerman & Kuhlman, 2000). In a study examining the relationship between personality traits and risk-taking behaviours in college students, males demonstrated higher risk-taking than females and these gender difference were largely mediated by

the personality trait of novelty seeking (M. Zuckerman & Kuhlman, 2000). In addition to these findings, which were based on personality questionnaire data, the results of Chapter Four show neural gender differences in reward processing, with boys showing significantly higher activation of the VS relative to girls. Although results suggest that gender differences in both novelty seeking and reward processing, gender differences in the relationship between VS activation and novelty seeking seeking have not been investigated.

It has been hypothesised that dopaminergic genes on the X-chromosome, and particularly the *MAOA* gene may mediate gender differences in personality traits, including novelty seeking (Lentini, Kasahara, Arver, & Savic, 2012; Savic, 2010). In fact, a recent study suggests that the high activity variant of the *MAOA*-VNTR is associated with both higher levels of novelty seeking and reward dependence, measured by the Temperament and Character Inventory (TCI) in a large sample of Japanese adults (n = 324) (Shiraishi et al., 2006).

When Cloninger and colleagues (1993) developed the TCI they hypothesised that the measures of novelty seeking and reward dependence would be associated with dopamine, serotonin and norepinephrine. Monoamine oxidase A (MAOA) is a mitochondrial enzyme involved in the degradation of the monoamines mentioned above. It has been reported that *MAOA* knockout mice exhibit increases in brain levels of dopamine, serotonin and norepinephrine, and increased aggressive and impulsive behaviours (Shih & Thompson, 1999). *MAOA* is also an X-linked gene which is suggested to account for gender differences in novelty seeking and impulsive behaviours. The fact that boys only have one copy of genes on the X-chromosome, such as *MAOA*, may make them more vulnerable to traits and disorders resulting from reduced expression of the gene.

Two recent studies have investigated the effect of *MAOA* on brain function in both males and females (Buckholtz, et al., 2008; Meyer-Lindenberg, et al., 2006). A study by Meyer-Lindenberg and colleagues revealed that the effect of *MAOA* was particularly pronounced in the anterior cingulate during response inhibition. These effects were identified only amongst males. The finding suggests that men carrying the low expression allele of *MAOA* (*MAOA*-L) are at increased risk to develop a neural phenotype associated with impulsive aggression. While this study identified associations between *MAOA* genotype and brain activation, the authors did not test for associations with behaviour. The second study by Buckholtz and colleagues showed that males carrying the low expression allele of the *MAOA*-VNTR showed reduced functional connectivity between the ventromedial prefrontal cortex and amygdala during a face processing task (Buckholtz, et al., 2008). However, the reduction in connectivity was not observed amongst women. Effect of *MAOA* on VS activation has not been tested during reward processing tasks or any other neuroimaging task. Based on these studies *MAOA* may be a candidate gene underlying gender specific brain function.

In this chapter we investigated whether there is a significant association between novelty seeking and VS activation during reward anticipation. We also explored whether *MAOA* genotype was associated with scores of novelty seeking and whether *MAOA* genotype differently affects VS activation during reward anticipation in adolescent boys and girls. In order to do so we measured ventral striatal activation and novelty seeking scores in adolescent boys and girls (n = 411) from the IMAGEN sample.

5.3 MATERIALS AND METHODS

5.3.1 PARTICIPANTS

We used data from the first wave of IMAGEN (n = 705). Individuals who had completed the MID task (n = 595), passed task specific outlier criteria, particularly in terms of movement (n = 516) and contrast-specific spike detection across voxels (n = 495), had been able to complete the task satisfactorily in the scanner (n = 493), had complete handedness data (n = 487), had complete IQ data and an verbal and reasoning IQ score > 75 (n = 464), had complete quality-controlled genetic data for the *MAOA*-gene (n = 427), did not show structural abnormalities (n = 423), had complete and quality control rated data on the Temperament and Character Inventory (n = 411) were included in the dataset. Thus, 411 adolescents survived the criteria (186 boys, 225 girls). The sample had a mean age of 14.4 years (SD: 0.4; range: 13.2-16.0 years) (see ***Table 9*** for demographics). Participants were tested in eight IMAGEN assessment centres (London, Nottingham, Dublin, Mannheim, Berlin, Hamburg, Paris and Dresden). The study was approved by local ethics research committees at each site. A detailed description of recruitment and assessment procedures, as well as in/exclusion criteria, has been described elsewhere (Schumann, et al., 2010). Three hundred and sixty two participants were right-handed and 49 participants were left-handed or ambidextrous. Individuals with verbal or performance IQ < 75 were excluded. Handedness and study site were controlled for in all analyses.

Table 9: Demographics split by gender and rs12843268 genotype groups: Means, standard deviations and ranges are presented below (Mean ± SD, *(Range)*). We found no significant genotype differences in age, verbal or performance IQ ($p > 0.05$) in boys or girls after controlling for study site.

	Boys			**Girls**			
	A N = 63	**G** N = 123	**Total** N = 186	**AA** N = 16	**AG** N = 102	**GG** N = 107	**Total** N = 225
Age (yrs)	14.5 ± 0.4 *(13.6-15.5)*	14.5 ± 0.4 *(13.6-15.6)*	14.5 ± 0.4 *(13.6-15.6)*	14.5 ± 0.4 *(13.9-15.6)*	14.4 ± 0.4 *(13.3-15.4)*	14.4 ± 0.5 *(13.3-15.5)*	14.4 ± 0.5 *(13.3-15.6)*
VIQ	117.3 ± 15.4 *(83-150)*	115.1 ± 14.6 *(87-155)*	115.7 ± 15.3 *(83-155)*	110.4 ± 11.8 *(88-130)*	112.6 ± 15.1 *(77-150)*	113.4 ± 14.9 *(77-152)*	112.6 ± 14.9 *(77-152)*
PIQ	107.7 ± 13.9 *(81-149)*	107.0 ± 12.5 *(79-135)*	106.8 ± 12.8 *(79-149)*	111.8 ± 12.9 *(92-141)*	111.4 ± 12.1 *(86-146)*	109.8 ± 12.8 *(76-135)*	110.8 ± 12.5 *(76-146)*
TCI Nov. Seeking	109.8 ± 10.9 *(88-142)*	110.7 ± 13.0 *(74-153)*	110.7 ± 12.4 *(74-153)*	107.7 ± 7.8 *(97-124)*	112.6 ± 13.4 *(79-143)*	111.3 ± 14.2 *(78-152)*	111.7 ± 13.5 *(78-152)*

TCI = Temperament and Character Inventory, VIQ = Verbal IQ, PIQ = Reasoning IQ,
Boys carry one A-allele or one G-allele, Girls are either AA homozygous, AG heterozygous or GG homozygous for rs12843268

5.3.2 TEMPERAMENT AND CHARACTER INVENTORY (TCI)

We used a shortened version of the TCI, specifically targeting the Novelty Seeking Scale which is combined of 4 subscales: i) Exploratory Excitability vs. Rigidity; ii) Impulsiveness vs. Reflection; iii) Extravagance vs. Reserve; iv) Disorderliness vs. Regimentation. This study used the self-ratings of the participants (see **Section 2.3.5**).

5.3.3 MONETARY INCENTIVE DELAY (MID) TASK

The participants performed a modified version of the MID task to study neural responses to reward anticipation and reward feedback (see Appendix 6 for second level model and random effects analysis for the reward anticipation contrast). The paradigm has been described in a previous publications (Nees, et al., 2012)(or see **Section 2.4.1**).

5.3.4 FMRI DATA ACQUISITION AND ANALYSIS

Structural and functional MRI data were acquired at eight IMAGEN assessment sites with 3T MRI scanners of different manufacturers. Functional MRI data was analysed using SPM-8 (Statistical Parametric Manual, 8th edition, http://www.fil.ion.ucl.ac.uk/spm) as described in **Section 2.4.3**. In the second level analysis (SPM-design: one-sample t-test) of anticipation large win vs. no win the following covariates were added to the second-level model: dummy-coded centre effects for the eight centres, handedness (right/ambidextrous) and gender (see ***Supplementary Figure 5***). In order to test the hypothesis that *MAOA* genotype affects VS activation differently in boys and girls we extracted the VS ROIs using the Marsbar toolbox (http://marsbar.sourceforge.net). The ROI for the VS was based on the ventral striatal peak from contrast 'anticipation of high win vs. anticipation of no win' of the IMAGEN sample (see **Chapter 3**). A sphere of 9 mm was drawn around this peak (xyz = ±9, 11, -2, radius of 9 mm).

5.3.5 GENOTYPING

DNA purification and genotyping was performed by the Centre National de Génotypage in Paris. DNA was extracted from whole blood samples (~10ml) preserved in BD Vacutainer EDTA tubes (Becton, Dickinson and Company, Oxford, UK) using Gentra Puregene Blood Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions. Genotype information was collected at 582,982 markers using the Illumina HumanHap610 Genotyping BeadChip (Illumina, San Diego, CA, USA) as part of a previous genome-wide association study (GWAS) (Schumann, et al., 2010) (see **Section 2.5**). Eight SNPs within the *MAOA* gene and promoter region (ChrX: 43395353-43491012) were targeted by Illumina HumanHap610 (see ***Figure 11***).

5.3.6 EFFECT OF *MAOA* RS12843268 ON MAOA EXPRESSION

Total RNA was extracted from whole blood cells using the PAXgene Blood RNA Kit (QIAGEN Inc., Valencia, CA, USA). Following quality control, labelled complementary RNA (cRNA) from n = 171 boys and n = 198 girls, was generated using the Illumina® TotalPrep™ RNA Amplification kit (Applied Biosystems/Ambion, Austin, TX, USA). *MAOA* expression was independently validated in boys using quantitative PCR (qPCR). Full details of expression analysis and qPCR are available in the **Section 2.6**.

5.3.7 ASSOCIATION ANALYSES

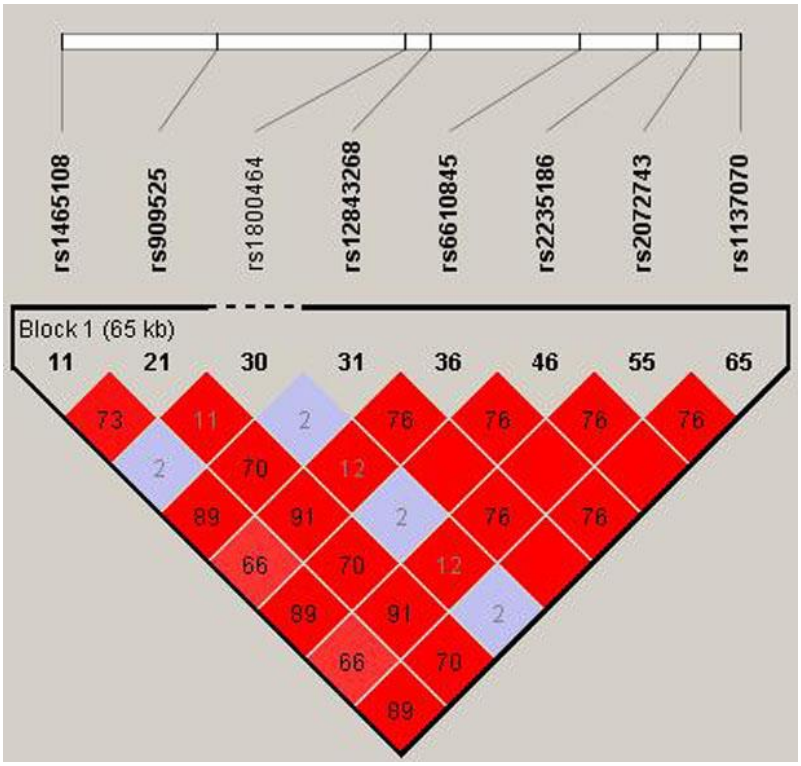
Partial correlations were performed to determine whether VS BOLD-responses are correlated with novelty seeking. The general linear model was used to determine associations between VS BOLD-responses and *MAOA* genotype in boys and girls separately. All analyses were two-sided.

5.4 RESULTS

5.4.1 IDENTIFICATION AND CHARACTERIZATION OF *MAOA* SNP

We extracted eight SNPs covering *MAOA* (***Figure 12***). The eight SNPs were in high linkage disequilibrium. Among the SNPs was rs12843268, which has previously been associated with externalising behaviours in the literature. It was also found that rs12843268 was expressed in our sample whereas the other seven polymorphisms did not show significant expression levels of MAOA. When we divided the sample by gender we found that the gene expression levels of rs12843268 were only significant in boys (t = 7.82, p = 0.006), but not in girls (t = 0.58, p = 0.45). In boys, the association was independently validated through qPCR, which showed a relative fold change in expression between the two genotypes of 6.34 (standard error [SE]: 0.296).

Figure 12. Plot of linkage disequilibrium and r^2-values of the eight *MAOA* SNPs covered by Illumina HumanHap610 BeadChip



5.4.2 Correlation between VS activation and novelty seeking

The correlations between left/right VS activation and TCI novelty seeking scores were not significant in the full sample (Left VS: r = -0.041, p = 0.414; Right VS: r = -0.048, p =0.342. After the sample was split by gender, the correlations between left/right VS activation and TCI novelty seeking scores were not significant amongst boys (Left VS: r = -0.044, p = 0.562; Right VS: r = -0.040, p =0.594) or girls (Left VS: r = -0.037, p = 0.591; Right VS: r = -0.069, p = 0.309).

5.4.3 Association between *MAOA* rs12843268 and novelty seeking

We were not able to replicate the relationship between *MAOA* genotype and novelty seeking (Shiraishi, et al., 2006). The association between *MAOA* SNP rs12843268 and novelty seeking was not significant in boys (t = 0.003, p = 0.956) or in girls (t = 0.547, p = 0.579).

5.4.4 Association between *MAOA* rs12843268 and VS activation

In order to investigate whether *MAOA* SNP rs12843268 mediates gender differences in VS activation, we explored the effect of the polymorphism in boys and girls separately.

5.4.4.1 Boys

In a sample of n = 186 boys (G hemizygotes: n = 123, A hemizygotes: n = 63) we found that *MAOA* genotype was significantly associated with VS BOLD-response, with G hemizygous boys showing significantly higher activation in the left VS (t = 9.37, p = 0.003, partial eta squared: 0.051) and right VS (t = 6.89, p = 0.009, partial eta squared: 0.038) relative to A hemizygous boys (***Figure 13*** and ***Figure 14***).

Figure 13. Genotype differences in left VS activation during reward anticipation amongst boys (t = 9.37, p = 0.003, partial eta squared: 0.051).

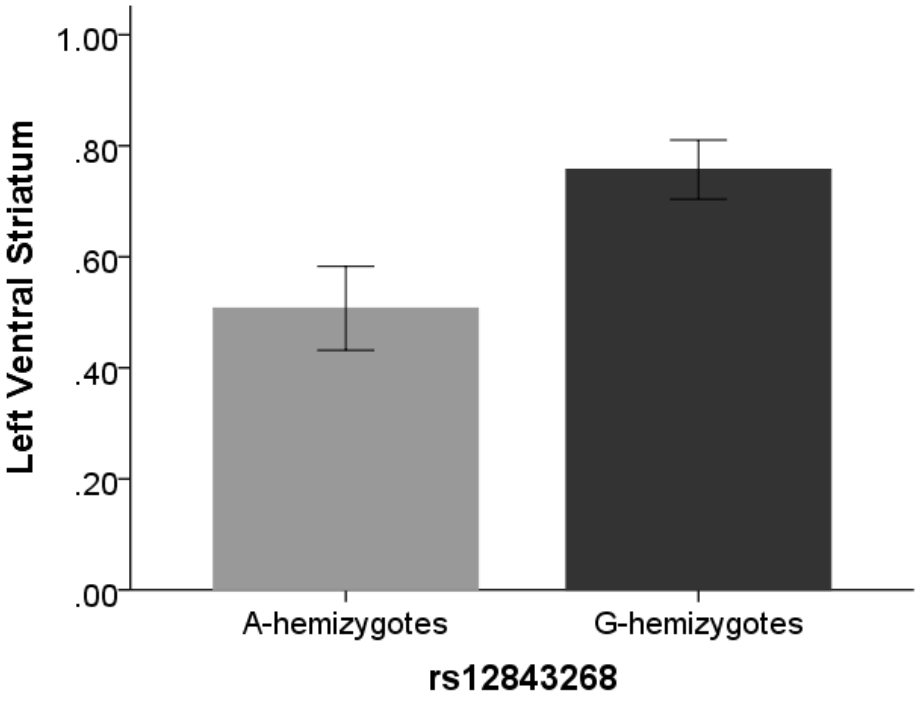
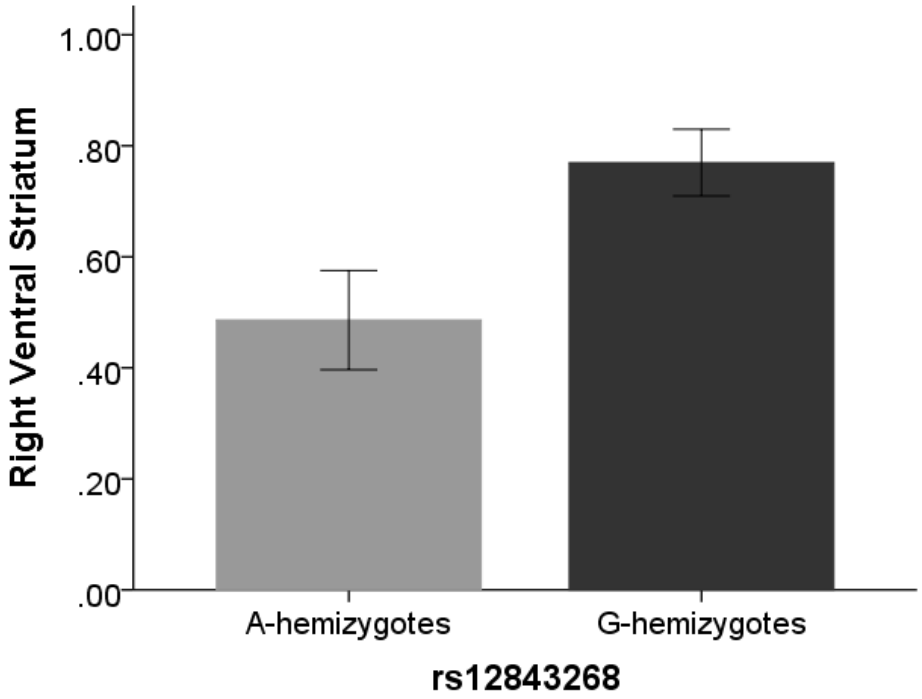


Figure 14. Genotype differences in right VS activation during reward anticipation amongst boys (t = 6.89, p = 0.009, partial eta squared: 0.038).



5.4.4.2 Girls

We investigated whether *MAOA* genotype was associated with left and right VS BOLD-response during reward anticipation in a sample of n = 225 (AA = 16, AG = 102, GG =107). We tested the additive model to determine whether *MAOA* genotype affects VS activation amongst girls. No significant associations were identified in the left VS (t = 0.56, p = 0.574) or right VS (t = 0.37, p = 0.691). Simple effects between the genotype groups suggest no significant association with VS activation (***Figure 15*** and ***Figure 16***).

Figure 15. Genotype differences in left VS activation during reward anticipation amongst girls (non-significant, $t = 0.56$, $p = 0.574$).

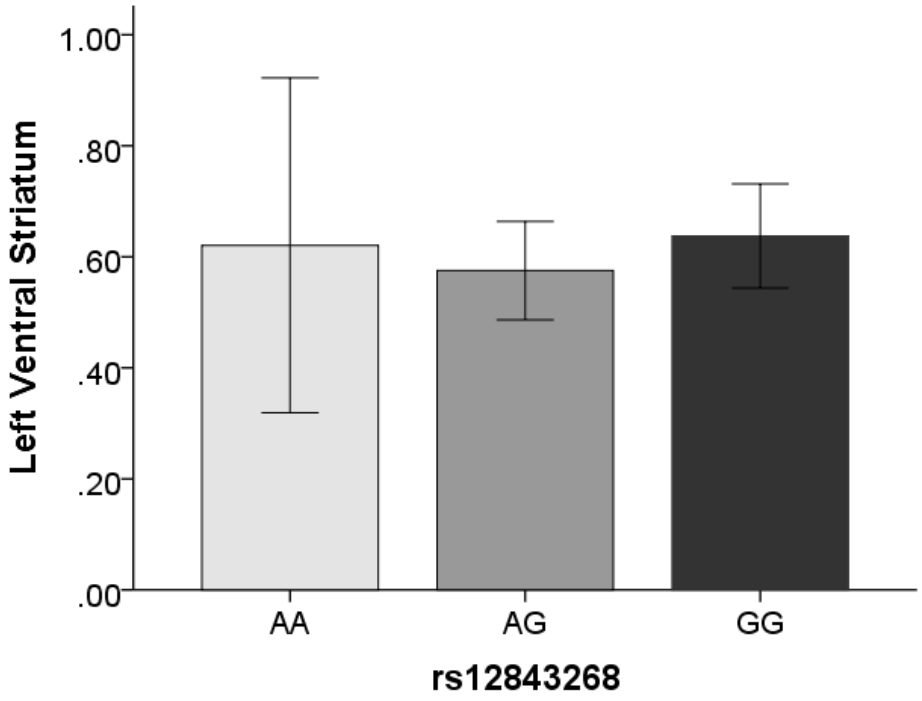
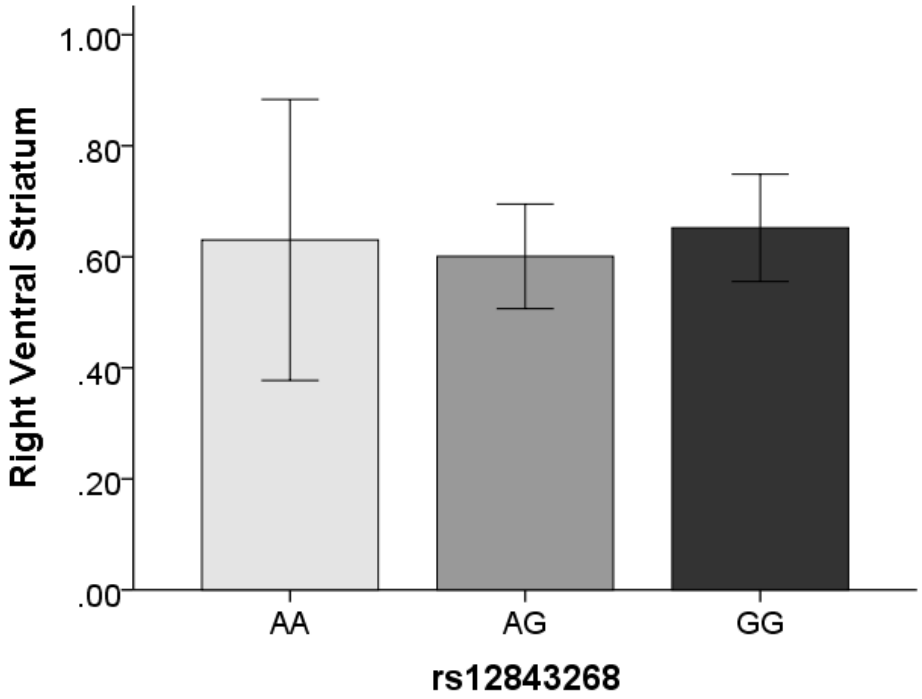


Figure 16. Genotype differences in right VS activation during reward anticipation amongst girls (non-significant, $t = 0.37$, $p = 0.691$).



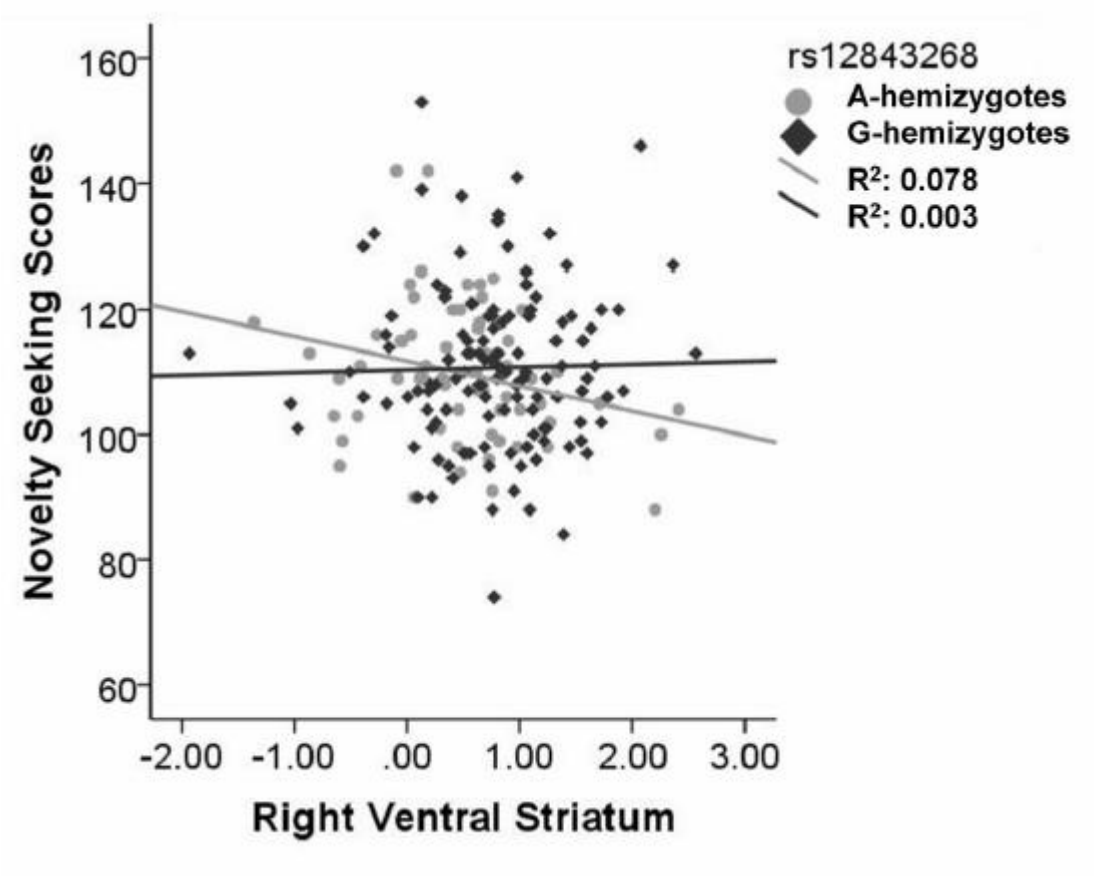
5.4.5 Association between VS activation and novelty seeking by genotype group

5.4.5.1 *Boys*

Considering that *MAOA* rs12843268 appears to affect VS BOLD-response in boys we investigated whether the correlation between VS BOLD-response and novelty seeking differed depending on *MAOA* genotype. We found a trend towards a right VS x *MAOA* interaction on novelty seeking scores in boys ($t = 3.1$, $p = 0.082$). When dividing the boys into groups depending on their *MAOA* genotypes we found a negative correlation between novelty seeking and VS BOLD-response in the A hemizygotes who initially showed reduced activation of the right VS ($r = -0.28$, $p = 0.038$), but not in the left VS ($r = -0.20$, $p = 0.145$) (***Figure 17***). No significant

correlation was found between novelty seeking and VS BOLD-response in G hemizygotes (right VS: r = 0.05, p =0.590; left VS: r = 0.02, p = 0.850).

Figure 17. Correlation between right VS activation and novelty seeking scores in boys: Right VS activation was negatively correlated with novelty seeking scores in A hemizygotes (r = -0.28, p = 0.038). The correlation was not significant in G hemizygotes (r = 0.05, p = 0.590).



5.4.5.2 *Girls*

No significant correlations between novelty seeking and VS activation were identified when girls were divided by *MAOA* genotype (AA: Left VS: r = -0.382, p = 0.276, Right VS: r = -0.303, p = 0.395; AG: Left VS: r = -0.046, p = 0.658, Right VS: r = -0.039, p = 0.711; GG: Left VS: r = -0.045, p = 0.661, Right VS: r = -0.119, p = 0.240).

5.5 DISCUSSION

This study investigated the relationship between novelty seeking and VS activation and explored whether this relationship differed depending on genetic variation in *MAOA* SNP rs12843268.

5.5.1 ASSOCIATION BETWEEN NOVELTY SEEKING AND VENTRAL STRIATAL ACTIVATION

Previous neuroimaging studies suggest a link between novelty seeking and reward processing in adolescence. Functional MRI studies indicate that novelty seeking behaviours during adolescence stem from immaturities in brain circuitry mediating reward processing. Several studies have supported this notion (Peters, et al., 2011; Wittmann, Daw, Seymour, & Dolan, 2008); however we were unable to replicate this association. The lack of association between VS activation and novelty seeking in our sample may be due to the use of a different questionnaire compared to previous studies. Some studies have used questionnaires that measure concrete behaviour (such as illicit drug use or heavy drinking) whereas others have investigated risk-taking bias using the neuropsychological Cambridge Guessing Task (Galvan, et al., 2007; Schneider, et al., 2012). The TCI is a personality questionnaire which asks participants to rate statements which may be less specifically phrased than those used by previous studies. However, it should be noted that one recent study did identify significant correlations between one TCI novelty seeking subscale (exploratory excitability) and activation of the ventral tegmental area/substantia nigra (VTA/SN) during reward anticipation (not measured by the MID task). However, we did not replicate this result (Krebs, et al., 2009). The exploratory excitability subscale was not associated with nucleus accumbens activation in the study by Krebs and colleagues.

5.5.2 THE EFFECT OF *MAOA* ON NOVELTY SEEKING

MAOA is frequently linked to novelty seeking and impulsive behaviours, which are behaviours that are more often observed in boys than girls. A study by Shiraishi et al. (2006) suggested that *MAOA* is associated with novelty seeking measured by the TCI. We did not confirm this finding. Although we used the same measure as Shiraishi and colleagues there are a number of differences between the studies which may account for the lack of replication. Shiraishi and colleagues studied novelty seeking in adults (mean age: 29.9) whereas we investigated novelty seeking in adolescents (mean age: 14.4). Whereas Shiraishi and colleagues used a Japanese sample, our participants were mainly of European origin. Thus, cultural response biases or ethnic differences in genetic make-up may account for the observed differences in results.

5.5.3 THE EFFECT OF *MAOA* ON VENTRAL STRIATAL ACTIVATION

MAOA is a promising candidate gene underlying gender differences in brain function. Buckholtz and colleagues showed that males carrying the low expression allele of the *MAOA*-VNTR showed reduced functional connectivity between the ventromedial prefrontal cortex and amygdala during a face processing task (Buckholtz, et al., 2008). However, the reduction in connectivity was not observed amongst women. Similarly, Meyer-Lindenberg and colleagues revealed a deficit in anterior cingulate BOLD-response during response inhibition amongst men carrying the low expression allele of the *MAOA*-VNTR (Meyer-Lindenberg, et al., 2006). This relationship was not identified amongst females.

We add to the previous literature by suggesting that A-hemizygous boys of rs12843268 show reduced activation of the VS during reward processing. However, this effect is not observed in girls. Thus, imaging genetic studies suggests that the

MAOA modulates brain activation in boys, but not in girls. This result may be explained by the finding that rs12843268 was not expressed in girls.

5.5.4 *MAOA* STRATIFICATION

We explored the relationship between the X-linked *MAOA*, reward processing and behaviours in boys and girls separately to determine whether *MAOA* has different effect on boys who carry only one copy of rs12843268, relative to girls. Boys, who are hemizygous for the A-allele of rs12843268, showed a negative correlation between VS activation and novelty seeking. However, *MAOA* had no effect on the relationship between novelty seeking and VS activation in girls. Girls may be protected against the negative effects of low MAOA expression by carrying two alleles of the gene. A-hemizygous boys, who only carry one allele of the rs12843268 may be unable to compensate for the reduced expression of the polymorphism. It is also worth noting that very few girls in our sample were homozygous for the minor allele of rs12843268 (n = 16). Thus, the sample may not be large enough to properly investigate the effect of the minor allele in girls.

In order to better understand the implications of our findings, we investigated the expression levels of MAOA in blood for boys and girls separately. We found that SNP rs12843268 is significantly expressed in boys, but not in girls. Gender differences in MAOA expression levels may be due to X-inactivation of the SNP, but previous studies also suggest that *MAOA* interacts with testosterone in cerebrospinal fluid, rendering males more sensitive to the effects of MAOA (Sjoberg, et al., 2008). Gender differences in VS activation may be the result of an MAOA x testosterone interaction, but this is in need of further investigation. *MAOA* is sometimes referred to as the ‘warrior gene’ due to its effect on impulsive and aggressive behaviour. Although the name ‘warrior gene’ is not fully deserved, it suggests that *MAOA*

represents one genetic variant that makes boys more likely to display aggressive and impulsive behaviours and disorders relative to girls.

We found that A hemizygous boys show reduced expression of *MAOA* as measured by presence of the MAOA enzyme in blood, compared to G hemizygotes. This suggests that A hemizygotes may not degrade catecholamines, such as dopamine, serotonin and norepinephrine, as efficiently as G hemizygotes. The A hemizygotes who showed reduced expression of MAOA also showed reduced VS activation, which was negatively correlated with novelty seeking.

5.5.5 LIMITATIONS

This study is subject to a couple of limitations. Firstly, it should be noted that whereas the association between *MAOA* and VS activation was not significant in girls, only a very small number of A homozygous girls were available in our sample. This number may have been too small to detect the correlations that were identified amongst the boys. However, if these results are confirmed in a larger population, the low number of A hemizygous girls in this population-based cohort may explain why novelty seeking behaviours are less frequently displayed in girls.

Secondly, this study investigates the relationship between *MAOA* and reward processing in adolescents. Prior studies suggest that the reward system undergoes substantial re-organisation during adolescence, during which hormones also have different effects in boys and girls. Thus, we are unable to conclude whether the observed gender differences would remain in an adult sample.

5.5.6 CONCLUSION

This study confirmed previous results which suggest that *MAOA* is a viable candidate underlying gender differences in brain function. Our findings also suggest that stratification by *MAOA* genotype may help explain the relationship between VS activation and novelty seeking behaviour in boys. Future work is necessary to fully understand the mechanisms by which *MAOA* modulates VS activation in boys.

6 CHAPTER SIX:
NEURAL MECHANISMS OF ADHD SYMPTOMS ARE STRATIFIED BY *MAOA* GENOTYPE

6.1 OBJECTIVES OF THIS CHAPTER

The objective of this chapter is to examine whether Monoamine Oxidase A (*MAOA)* genotype is associated with symptoms of Attention Deficit Hyperactivity Disorder (ADHD) and the neural mechanisms of reward processing and inhibitory control believed to contribute to ADHD symptoms. The specific aims of this chapter are as follows:

1. Determine whether *MAOA* genotype is associated with ADHD symptoms in boys and girls
2. Replicate previous studies which suggest that ADHD symptoms are correlated with VS activation (measured during MID) and right IFG activation (measured during SST)
3. Investigate whether *MAOA* genotype is associated with ventral striatal (VS) activation and right inferior frontal (IFG) activation
4. Explore the expression levels of the two different alleles of *MAOA* rs12843268
5. Determine whether *MAOA* stratifies the relationship between ADHD symptoms and brain activation patterns in the VS and right IFG

6.2 INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a heritable disorder (Faraone & Doyle, 2001), characterised by symptoms of inattention, hyperactivity and impulsivity (American Psychiatric Association, 2000). ADHD symptoms are more common in males than females with gender ratios varying from 3:1 to 9:1 (Arnold, 1996; Gaub & Carlson, 1997). Given these gender differences it has been suggested that genes on the X-chromosome may be responsible for the development of ADHD.

MAOA, which is located on the X-chromosome between p11.23 and p11.4, is one candidate gene that may mediate gender differences in personality and psychiatric disorders. *MAOA* encodes a mitochondrial enzyme, which degrades monoamines, including norepinephrine, dopamine and serotonin (Shih, 2004). The gene is a candidate for ADHD because it influences the monoaminergic systems, which are thought to underlie the neural functions associated with the disorder. Several studies have identified associations between specific *MAOA* polymorphisms and ADHD (Brookes et al., 2006; Das et al., 2006; Domschke et al., 2005; Guan, et al., 2009). Most recently, a screen of 23 candidate genes believed to contribute to ADHD (including *COMT DRD1-DRD4, DAT1, SNAP25, MAOA* and *MAOB*) suggested that *MAOA* is the most promising candidate gene underlying ADHD (Guan, et al., 2009). Out of 12 *MAOA* polymorphisms which were tested for association with ADHD, rs12843268 showed the strongest association. Another study which aimed to determine the effect of *MAOA* on neuropsychological functioning in ADHD suggested that a haplotype including rs12843268 was associated with poorer motor functioning in boys with ADHD (Rommelse, et al., 2008). In addition to these studies, a variable tandem repeat (VNTR) within the promoter of *MAOA* is frequently linked with ADHD (Manor et al., 2002). The VNTR has also been associated with

inhibitory control (Meyer-Lindenberg, et al., 2006) and novelty seeking (Bodi et al., 2009; Shiraishi, et al., 2006).

Studies of the association between *MAOA* and ADHD have focused predominantly on boys. Only one study separately analysed results from a small sample of girls with ADHD (n = 19) and a larger sample of boys with ADHD (n = 110). The study reported that *MAOA* did not have a significant effect on ADHD in girls (Manor, et al., 2002).

MAOA is known to affect ADHD; however it is unknown whether *MAOA* affects the neural mechanisms which have been associated with ADHD. *MAOA* is known to affect dopaminergic and serotonergic pathways, involved in reward processing and inhibitory control. A study by Meyer-Lindenberg and colleagues (Meyer-Lindenberg, et al., 2006) suggest that *MAOA* is significantly associated with several neuroimaging phenotypes believed to underlie impulsivity and aggression. The authors examined the relationship between *MAOA*-VNTR genotype and brain function, but their study did not test whether *MAOA* or brain function were associated with a trait or disorder. The study found a significant association between *MAOA* genotype and anterior cingulate activation during inhibitory control. This relationship was only identified in males; no association between *MAOA* genotype and anterior cingulate activation was found in females.

The reward and inhibitory control systems are frequently investigated in ADHD. Several studies suggest that ADHD patients show reduced BOLD-response of the VS relative to healthy controls (Carmona, et al., 2011; Hoogman, et al., 2011; Scheres, et al., 2007b; Strohle, et al., 2008). Negative correlations between ADHD symptom-count and VS activation have been reported amongst ADHD patients (Scheres, et al., 2007b; Strohle, et al., 2008).

ADHD has also been associated with poor response inhibition resulting either from insufficient activation of the inferior frontal gyrus (IFG) (Dickstein, Bannon, Castellanos, & Milham, 2006; Rubia, 2011; Rubia, et al., 2005) or from a requirement for larger frontal recruitment for optimal task performance (Ma et al., 2011; Pliszka, et al., 2006; Schulz, et al., 2004; Schulz, Newcorn, Fan, Tang, & Halperin, 2005). Thus it appears that BOLD-responses of the subcortical reward system as well as inferior frontal inhibitory mechanisms, and particularly the right IFG, are crucially related to ADHD symptoms (Carmona, et al., 2011; Hampshire, et al., 2010).

Reward processing and response inhibition have never been tested together in adolescents with ADHD. Thus, it is unclear whether ADHD symptoms in the same individuals are associated with abnormalities in either or both systems. We therefore targeted both systems, and investigated potential determinants of brain activity in the regions involved. The *MAOA* gene is X-inactivated and highly methylated in females (Pinsonneault, Papp, & Sadee, 2006), which affects both tissue-specific allelic expression and gene expression. Therefore, we investigated the effect of *MAOA* in boys and girls separately.

In a large community-based sample of 414 adolescents from the IMAGEN study, we investigated whether *MAOA* genotype is associated with ADHD symptoms. We then carried out stratified analyses of performance and brain activation in the key reward area of the VS and the principal inhibitory frontal area, the right IFG. On the basis of aetiological models introduced above, we hypothesised that there would be a) a significant association between ADHD symptoms and *MAOA* genotype in boys, b) a significant association between ADHD symptoms and VS and right IFG BOLD-responses, and c) that the association between ADHD symptoms and brain activation

patterns during reward processing and inhibitory control is stratified by *MAOA* genotype.

6.3 MATERIALS AND METHODS

6.3.1 PARTICIPANTS

We used data from the first wave of IMAGEN (n = 705). Individuals who had completed the MID task (n = 595), passed task specific outlier criteria, particularly in terms of movement (n = 516), passed contrast-specific outlier criteria in terms of spike detection control (n = 495), had been able to see the task in the scanner (n = 482), had complete handedness data (n = 476), had complete IQ data (n = 456), had complete genetic data (n = 418), did not show structural abnormalities (n = 415), had complete data on the SDQ (n = 414) were included in the dataset. Thus, 414 adolescents passed the inclusion criteria for further analysis (190 boys, 224 girls). The mean age of the participants were 14.4 years (SD: 0.4; range: 13.3-15.6 years) (see ***Table 10*** for demographics).

Out of the 190 boys who had completed the MID task, 143 had also completed the stop signal task. Demographics of the sample of n = 143 boys can be found in **Supplementary Table 2**. Participants were tested in eight IMAGEN assessment centres (London, Nottingham, Dublin, Mannheim, Berlin, Hamburg, Paris and Dresden). The study was approved by local ethics research committees at each site. A detailed description of recruitment and assessment procedures, as well as in/exclusion criteria, has previously been published elsewhere (Schumann, et al., 2010). Three hundred and sixty seven participants were right-handed and 47 participants were left-handed or ambidextrous. Individuals with verbal (VIQ) or performance (PIQ) IQ < 75 or IQ-information missing were excluded (n = 10). Handedness and study site were controlled for in all analyses.

Table 10: Demographics split by gender and rs12843268 genotype groups: Means, standard deviations and ranges are presented below (Mean ± SD (*Range)*). We found no significant genotype differences in age, verbal or performance IQ ($p > 0.05$) in boys or girls after controlling for study site.

	Boys			**Girls**			
	A N = 67	**G** N = 123	**Total** N = 190	**AA** N = 16	**AG** N = 100	**GG** N = 108	**Total** N = 224
Age (yrs)	14.5 ± 0.4 *(13.6-15.5)*	14.5 ± 0.4 *(13.6-15.6)*	14.5 ± 0.4 *(13.6-15.6)*	14.5 ± 0.4 *(13.9-15.6)*	14.4 ± 0.4 *(13.3-15.4)*	14.4 ± 0.5 *(13.3-15.5)*	14.4 ± 0.04 *(13.3-15.6)*
VIQ	117.4 ± 14.9 *(83-150)*	115.1 ± 14.6 *(87-155)*	115.9 ± 14.7 *(83-155)*	110.4 ± 11.8 *(88-130)*	112.6 ± 15.1 *(77-150)*	113.1 ± 15.2 *(77-152)*	112.7 ± 14.9 *(77-152)*
PIQ	107.2 ± 13.7 *(81-149)*	107.0 ± 12.5 *(79-135)*	107.0 ± 12.9 *(79-149)*	111.8 ± 12.9 *(92-141)*	111.4 ± 12.1 *(86-146)*	109.3 ± 12.8 *(76-135)*	110.9 ± 12.7 *(76-147)*
ADHD Symptoms	2.7 ± 1.9 *(0-7)*	3.4 ± 2.6 *(0-10)*	3.1 ± 2.1 *(0-10)*	2.7 ± 1.9 *(0-7)*	2.3 ± 2.1 *(0-8)*	2.6 ± 2.2 *(0-10)*	2.4 ± 2.1 *(0-10)*

ADHD = Attention Deficit Hyperactivity Disorder, VIQ = Verbal IQ, PIQ = Reasoning IQ,
Boys carry one A-allele or one G-allele, Girls are either AA homozygous, AG heterozygous or GG homozygous for rs12843268

6.3.2 ADHD SYMPTOMS

ADHD symptoms were assessed using parental reports of the Strengths and Difficulties Questionnaire (SDQ), a brief 25-item behavioral screening tool probing for ADHD type problems (hyperactivity, inattention and impulsivity), emotional symptoms, conduct problems, peer problems and prosocial behavior (Herjanic & Reich, 1997). Our sample of n = 414, consisted of n = 28 subjects (20 boys) who were labelled as 'possibly' suffering from ADHD according to the SDQ. We identified a significant association between ADHD status and the level of ADHD symptoms in our sample ($t = 79.67$; $p < 0.0001$).

6.3.3 GENOTYPING

DNA purification and genotyping was performed by the Centre National de Génotypage in Paris. DNA was extracted from whole blood samples (~10ml) preserved in BD Vacutainer EDTA tubes (Becton, Dickinson and Company, Oxford, UK) using Gentra Puregene Blood Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions. Genotype information was collected at 582,982 markers using the Illumina HumanHap610 Genotyping BeadChip (Illumina, San Diego, CA, USA) as part of a previous genome-wide association study (GWAS) (Schumann, et al., 2010) (see **Section 2.5**). Eight SNPs within the *MAOA* gene and promoter region (ChrX: 43395353-43491012) were targeted by Illumina HumanHap610 (see ***Figure 12***).

6.3.4 EFFECT OF RS12843268 ON MAOA EXPRESSION

Total RNA was extracted from whole blood cells using the PAXgene Blood RNA Kit (QIAGEN Inc., Valencia, CA, USA). Following quality control, labelled complementary RNA (cRNA) from n = 171 boys and n = 198 girls, was generated using the Illumina® TotalPrep™ RNA Amplification kit (Applied Biosystems/Ambion, Austin, TX, USA). *MAOA* expression was independently validated in boys using quantitative PCR (qPCR). Full details of expression analysis and qPCR are available in the **Section 2.6**.

6.3.5 MONETARY INCENTIVE DELAY (MID) TASK

The participants performed a modified version of the MID task to study neural responses to reward anticipation and reward feedback see **Appendix 6** for second level model and random effects analysis of this contrast). The paradigm has been described in a previous publication (Nees, et al., 2012)(or see **Section 2.4.1**).

6.3.6 STOP SIGNAL TASK (SST)

Participants also performed an event-related SST designed to study neural responses to successful and unsuccessful inhibitory control (Rubia, et al., 2005; Rubia, et al., 2007). The task has been previously described in previous publications (Rubia, et al., 2005; Rubia, et al., 2007) (or see **Section 2.4.2**). In this study we used the contrast Stop success vs. Go success, which subtracts activation associated with Go success trials from the activation associated with Stop success trials (see **Appendix 6** for second level model and random effects analysis of the contrast). The dependent variable of the task is the stop signal reaction time (SSRT) which was calculated by subtracting the mean stop signal delay (SSD: the average time between Go and Stop signal, at which the subject managed to inhibit to 50% of trials) from the mean

reaction time (MRT) to Go trials (Logan, et al., 1997). Due to problems in the tracking algorithm, SSRT data was only available for n = 73 subjects.

6.3.7 FMRI DATA ACQUISITION AND ANALYSIS

Structural and functional MRI data were acquired at eight IMAGEN assessment sites with 3T MRI scanners of different manufacturers (Siemens, Philips, General Electric, Bruker). Functional MRI data was analysed with SPM-8 (Statistical Parametric Mapping, 8th edition, http://www.fil.ion.ucl.ac.uk/spm). In the second level analysis (SPM-design: one-sample t-test) of anticipation large win vs. no win and stop success vs. go success the following covariates were added to the second-level model: dummy-coded centre effects for the eight centres and handedness (right/ambidextrous) (see **Appendix 6**). In order to test the hypothesis that *MAOA* genotype affects VS and IFG activation we extracted regions of interest (ROIs) using the Marsbar toolbox (http://marsbar.sourceforge.net). The ROI for the VS was extracted based on the ventral striatal peak from contrast 'anticipation of high win vs. anticipation of no win' of the IMAGEN sample (xyz = ±9, 11, -2, radius of 9 mm). The IFG opercularis was extracted based on the MNI Automated Anatomical Labeling (AAL) (http://marsbar.sourceforge.net/). As the VS is not available as an AAL this was created using Marsbar. Further information is available in **Section 2.4.3**.

6.3.8 ASSOCIATION ANALYSES

The general linear model was used to determine associations between the SDQ measure, BOLD-responses and *MAOA* genotype. Correlations between fMRI BOLD-responses and SSRT were derived through Pearson correlations. All analyses were two-sided.

6.4 RESULTS

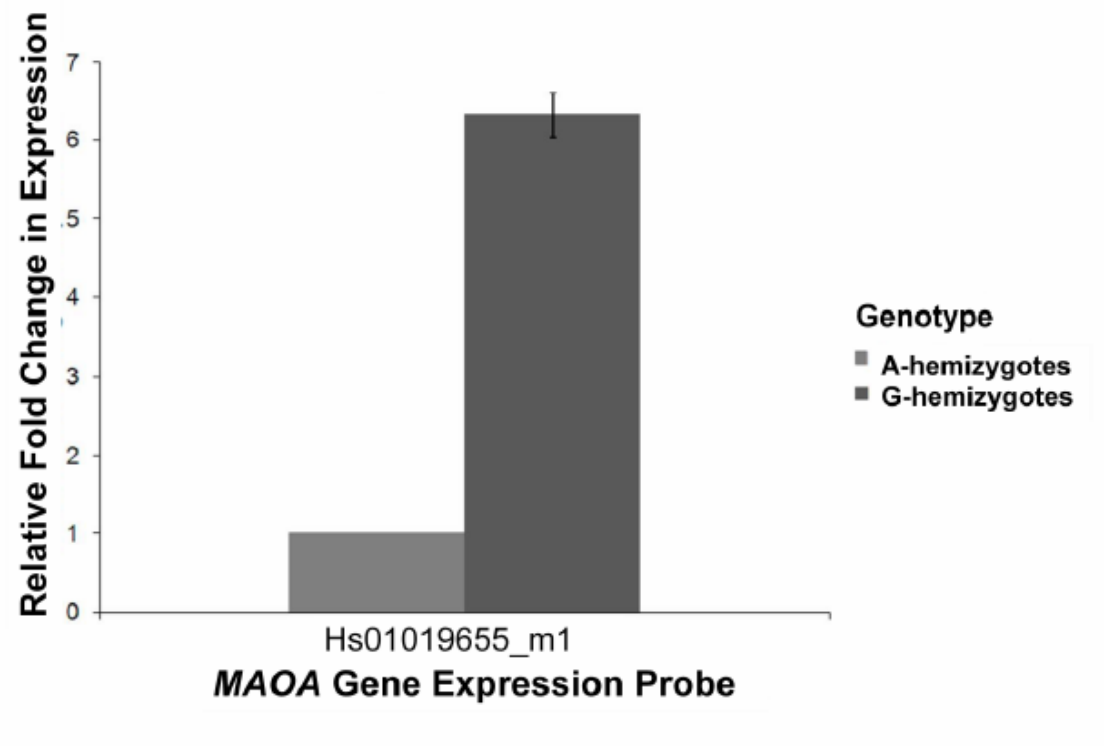
6.4.1 IDENTIFICATION AND CHARACTERIZATION OF *MAOA* SNP

We extracted eight SNPs covering the *MAOA* locus and identified two haplotypes with a frequency >5% which accounted for 92.3% of the variance of the gene (***Table 11*** and ***Figure 12***). Among the SNPs segregating the two haplotypes was rs12843268, which has previously been associated with ADHD symptoms (Guan, et al., 2009; Rommelse, et al., 2008). We therefore selected rs12843268 for further analyses. G hemizygotes of rs12843268 represent the major haplotype with a frequency of 63.4% whereas A hemizygotes represent the minor haplotype with a frequency of 28.9%. Gene expression data of *MAOA* from peripheral blood were available from 171 boys and 198 girls of the IMAGEN sample. In boys, we found significant differences between genotype groups of rs12843268 ($t = 7.82$, $p = 0.006$), with higher *MAOA* messenger RNA (mRNA) expression in the G hemizygous boys compared to A hemizygous boys. The expression analysis was not significant in girls ($t = 0.58$, $p = 0.45$). The association was independently validated through quantitative PCR in RNA from 40 boys, which showed a relative fold change in expression between the two genotypes of 6.34 (standard error (SE): 0.296) (***Figure 18***).

Table 11: Haplotype analysis of *MAOA* gene: Tagging SNP rs12843268 segregates haplotypes with a frequency of >5% and accounts for 92.3% of the variance of the gene.

	rs1465108	***rs909525***	***rs1800464***	***rs12843268***	***rs6610845***	***rs2235186***	***rs2072743***	***rs1137070***	***p-value***	***Frequency***
Hap 1	1	1	1	1	1	1	1	1	0.016	0.634
Hap 2	0	0	1	0	0	0	0	0	0.063	0.289
Tot Freq										0.923

Figure 18. Genotype-specific expression of *MAOA*: We found a relative 6-fold change in expression associated with *MAOA* rs12843268 genotypes in boys. Data are shown for the *MAOA* TaqMan® probe Hs01019655_m1 compared to the expression of the calibrator 18S gene (Probe ID: HS 99999901_s1).



6.4.2 EFFECTS OF *MAOA* GENOTYPE ON ADHD SYMPTOMS

Due to the X-linked nature of *MAOA* we tested its association with ADHD symptoms in boys and girls separately. We found that *MAOA* SNP rs12843268 was significantly associated with ADHD symptoms in boys (t = 4.12, p = 0.044, partial eta squared: 0.022) with G hemizygotes (n = 123) showing a significantly higher level of ADHD symptoms compared to A hemizygotes (n = 67) **(*Figure 19*)**. Rs12843268 genotype accounted for 2.2% of the variance in ADHD symptoms. We did not find a significant association between *MAOA* SNP rs12843268 and ADHD symptoms in

girls (t = 1.47, p = 0.23) (***Figure 20***), suggesting that the effects of *MAOA* are gender specific. We performed imaging genetic analyses in boys only.

Figure 19. Association between *MAOA* genotype and ADHD symptoms in boys: We found a significant association between *MAOA* rs12843268 genotype and ADHD symptoms, indicating increased ADHD symptoms in G hemizygotes compared to A hemizygotes (t = 4.12, p = 0.044, partial eta squared: 0.022).

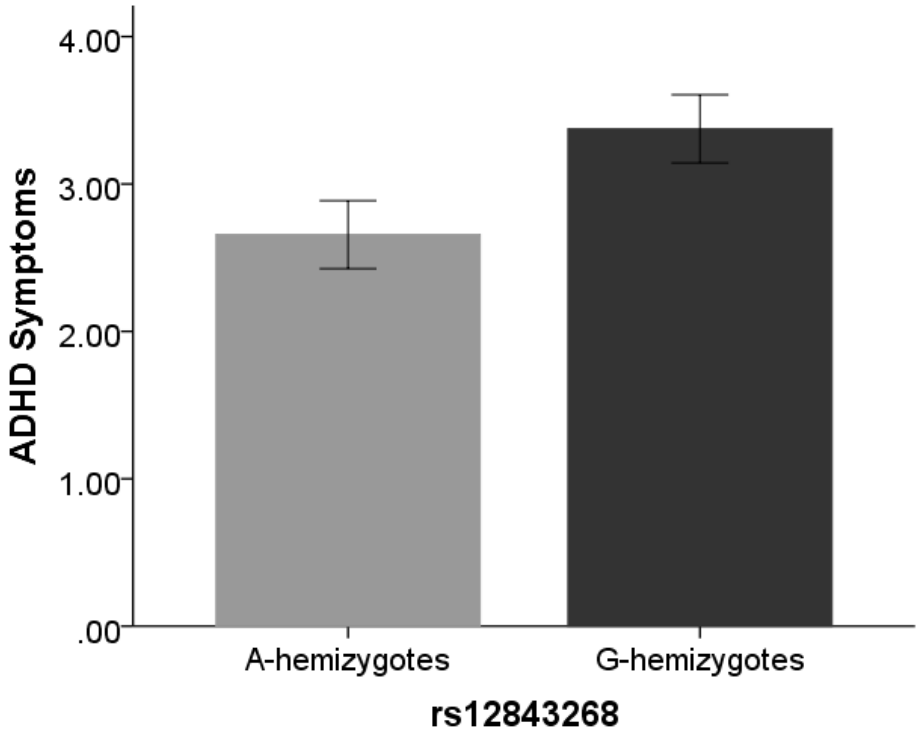
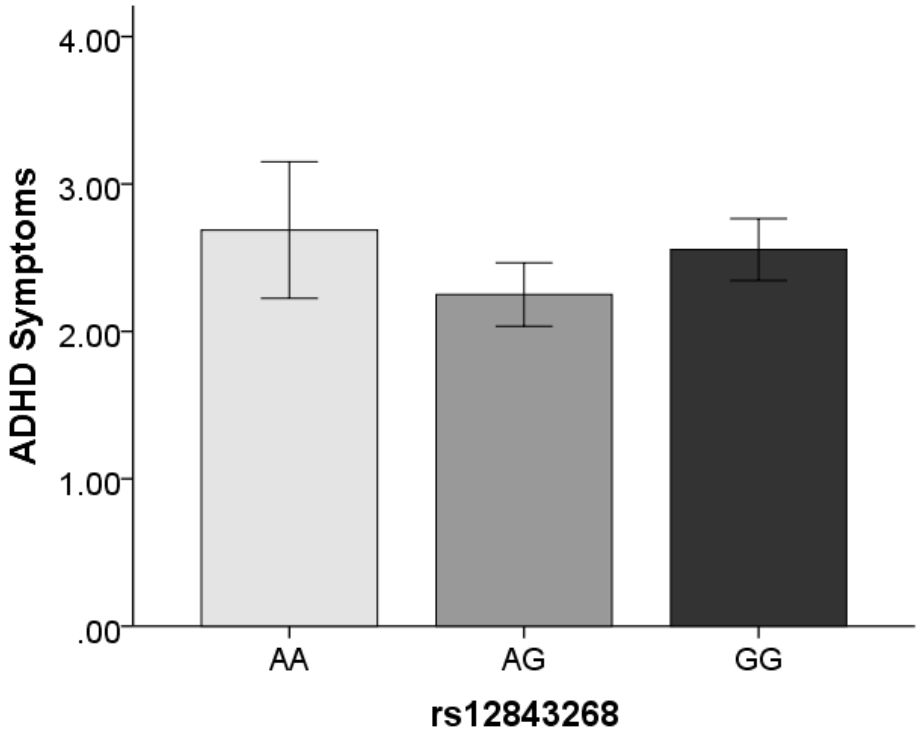


Figure 20. Association between *MAOA* genotype and ADHD symptoms in girls: We found no significant association between *MAOA* rs12843268 genotype and ADHD symptoms ($t = 1.47$, $p = 0.23$).



6.4.3 REWARD ANTICIPATION

6.4.3.1 Association between MAOA rs12843268 and VS activation in boys

As shown in Chapter Five we found that *MAOA* SNP rs12843268 was significantly associated with VS activation during reward anticipation. G hemizygotes showed significantly higher BOLD-response than A hemizygotes in the left VS ($t = 10.87$, $p = 0.001$, partial eta squared: 0.061; ***Figure 21***) and in the right VS ($t = 6.80$, $p = 0.007$, partial eta squared: 0.045; ***Figure 22***).

Figure 21. Associations between *MAOA* genotype and left VS activation: *MAOA* rs12843268 is associated with left VS activation ($t = 10.87$, $p = 0.001$, partial eta squared: 0.061).

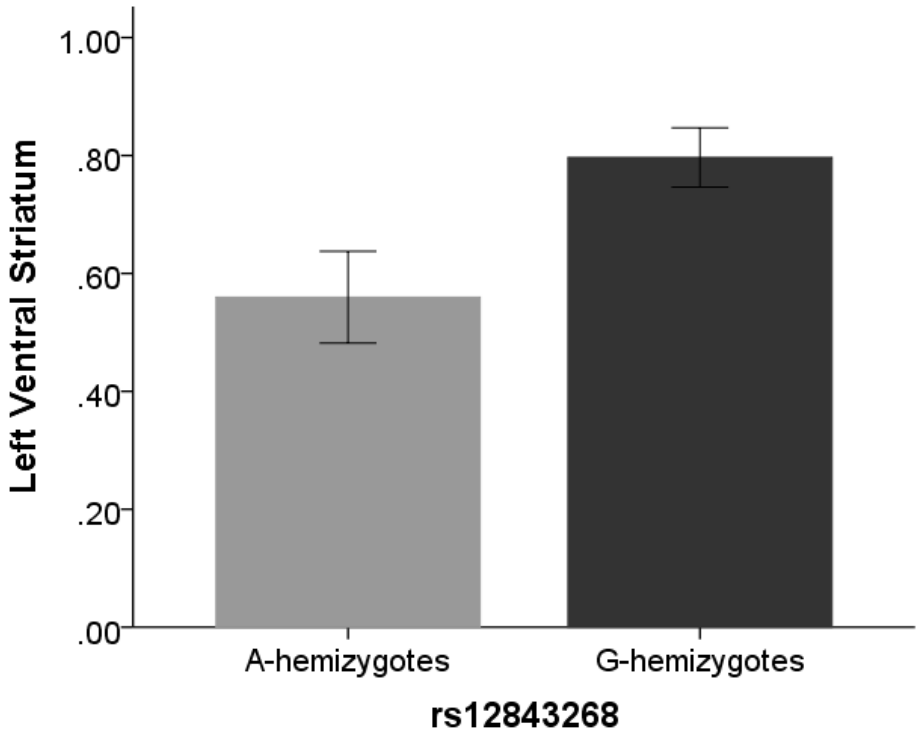


Figure 22. Associations between *MAOA* genotype and right VS activation: *MAOA* rs12843268 is associated with right VS activation (t = 6.80, p = 0.007, partial eta squared: 0.045) during reward anticipation.

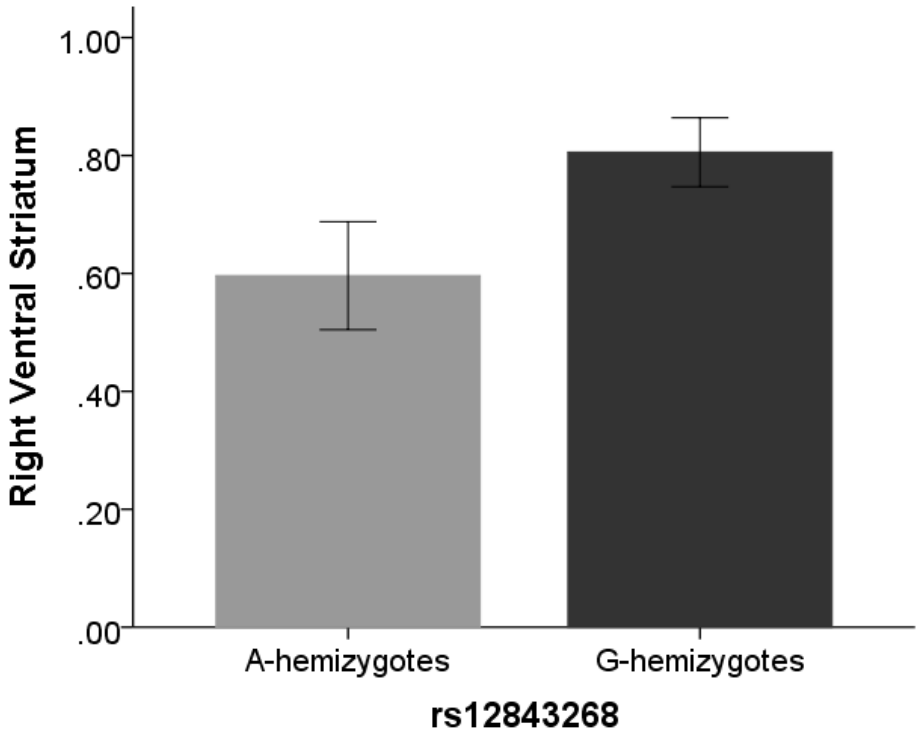
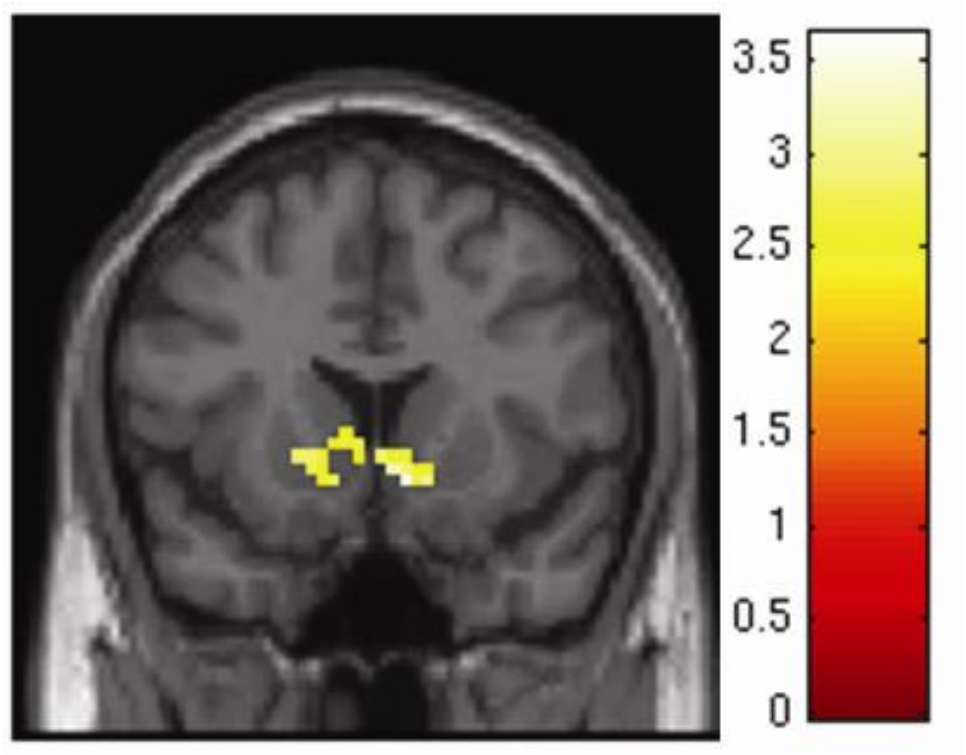


Figure 23. Coronal section showing genotype differences in VS activation during reward anticipation suggesting that G hemizygotes of *MAOA* rs12843268 show higher activation of bilateral VS compared to A hemizygotes (±9, 11, -2, 9mm radius sphere, $p < 0.01$, uncorrected).



6.4.3.2 Effect of MAOA rs12843268 on the relationship between VS activation and ADHD symptoms in boys

We next examined whether individual variability in VS BOLD-response were correlated with ADHD symptoms in boys. We found a nominally significant negative correlation between the right VS BOLD-response and ADHD symptoms in boys (r = -0.16, p = 0.035). When analyses were stratified by rs12843269 genotype we found that the negative correlation observed between right VS BOLD-response and ADHD symptoms was driven by A hemizygotes (right VS: r = -0.29, p = 0.025; left VS r = -0.22, p = 0.08). We observed no significant correlation between VS BOLD-responses

and ADHD symptoms in G hemizygotes (right VS: r = -0.15, p = 0.091; left VS: r = -0.14, p = 0.11) **(*Figure 24* and *Figure 25*)**. We also observed no significant genotype differences in reaction times (RT) during the MID task **(*Table 12*)**.

Figure 24. Correlation of VS activation and ADHD symptoms: The correlation between right VS activation and ADHD symptoms were driven by A hemizygotes (r = -0.29, p = 0.025). No significant association was found in the left VS or amongst G hemizygotes in either left or right VS.

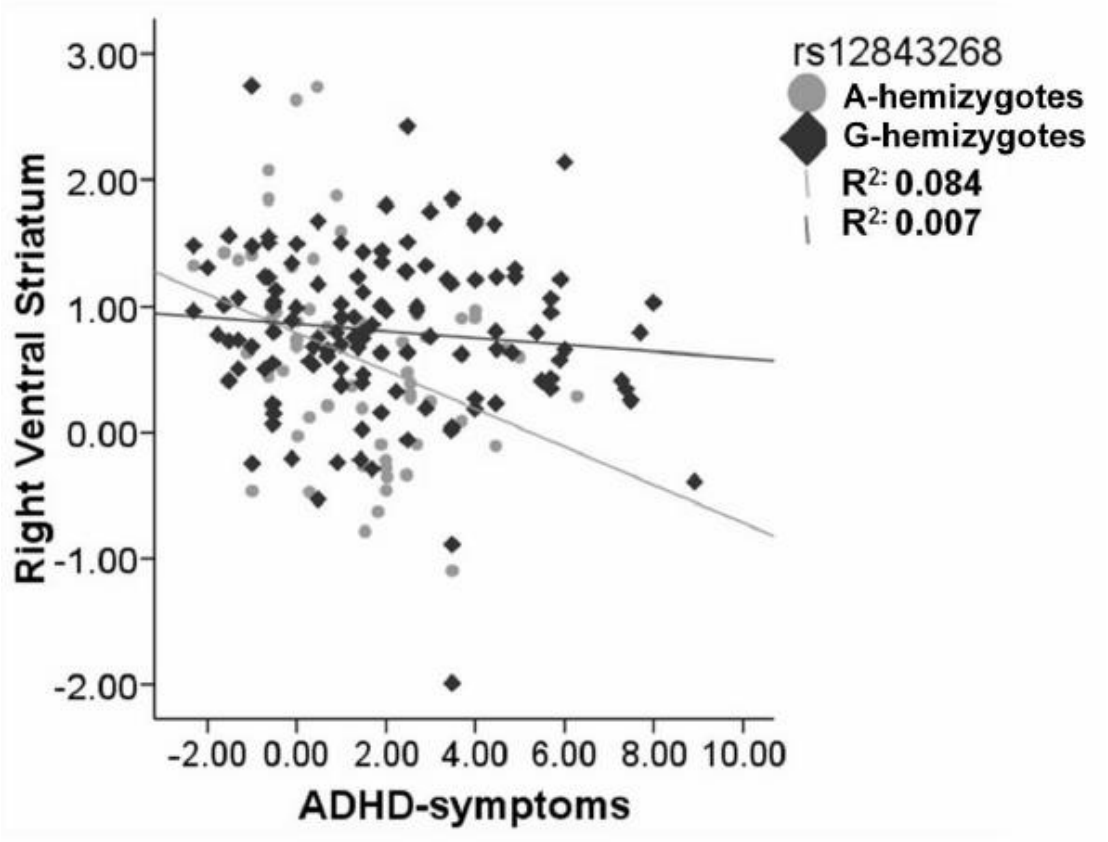


Figure 25. Coronal section showing the correlation between right VS activation and ADHD symptoms during reward anticipation in A hemizygotes (9, 11, -2, 9mm radius sphere, $p < 0.05$, uncorrected).

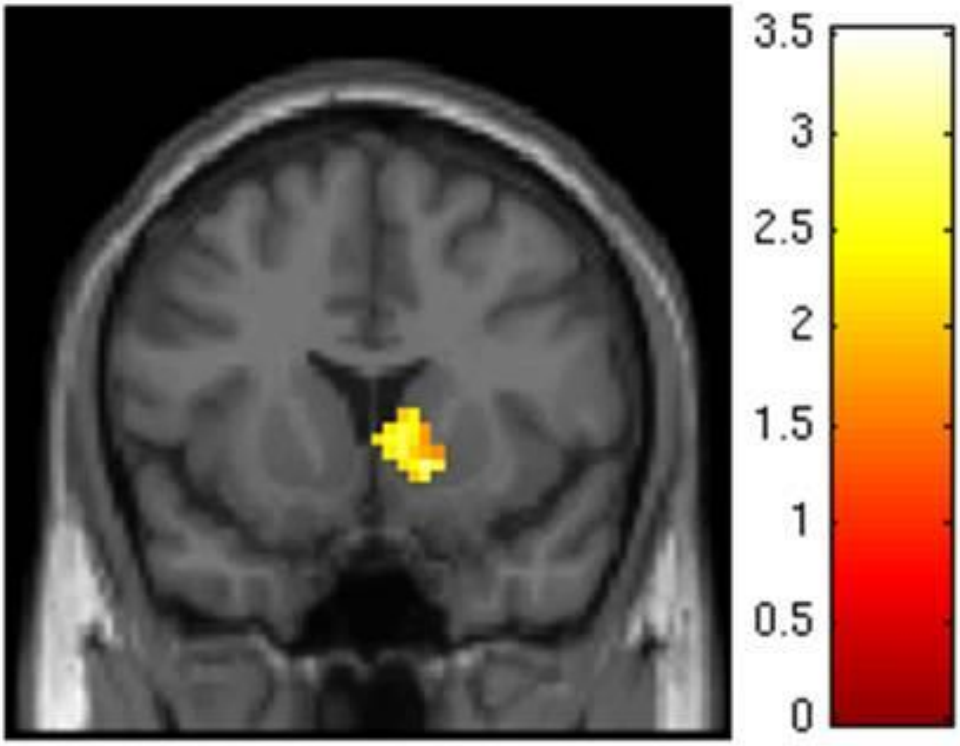


Table 12: Reaction times of responses during the MID task by rs12843268 genotype: Means and standard deviations are presented for reaction times (RT) measured during MID high win and no win trials. RTs suggest no significant rs12843268 genotype-differences in RT and no significant association between RTs and VS BOLD-responses during reward anticipation (data available for 163 out of 190 participants).

	A (n=56)	***G (n=107)***	***Full sample(n=163)***	***Genotype diff. in RT***	***Left VS/RT corr.***	***Right VS/RT corr.***
RT MID High Win	227.7 ± 18.8	228 ± 25.8	227.9 ± 23.6	t = 0.09, p = 0.93	r = -0.13, p = 0.12	r = -0.12, p = 0.12
RT MID No Win	236.5 ± 24.1	236.7 ± 25.1	236.6 ± 24.7	t = 0.05, p = 0.96	r = -0.03, p = 0.68	r = -0.03, p = 0.70

RT: Reaction time, MID: Monetary Incentive Delay, VS: Ventral Striatum

Without rs12843268 stratification the neural responses in the right VS accounted for 2.6% of the variance in ADHD symptoms, whereas after stratification 8.4% of the variance in ADHD symptoms was accounted for in A hemizygotes.

While the negative correlations between the right VS BOLD-response and ADHD symptoms is consistent with a blunted reward system (Scheres, et al., 2007b; Strohle, et al., 2008), the absence of a significant association between VS BOLD-response and ADHD symptoms in G hemizygotes suggested that brain regions other than the VS might mediate the effect of rs12843268 on ADHD symptoms in G hemizygotes. We hypothesised that G hemizygotes might show an association of response inhibition and ADHD symptoms (Hampshire, et al., 2010; Rubia, et al., 2005). Therefore, we investigated genotype specific BOLD-response of the right IFG during successful response inhibition.

6.4.4 SUCCESSFUL RESPONSE INHIBITION

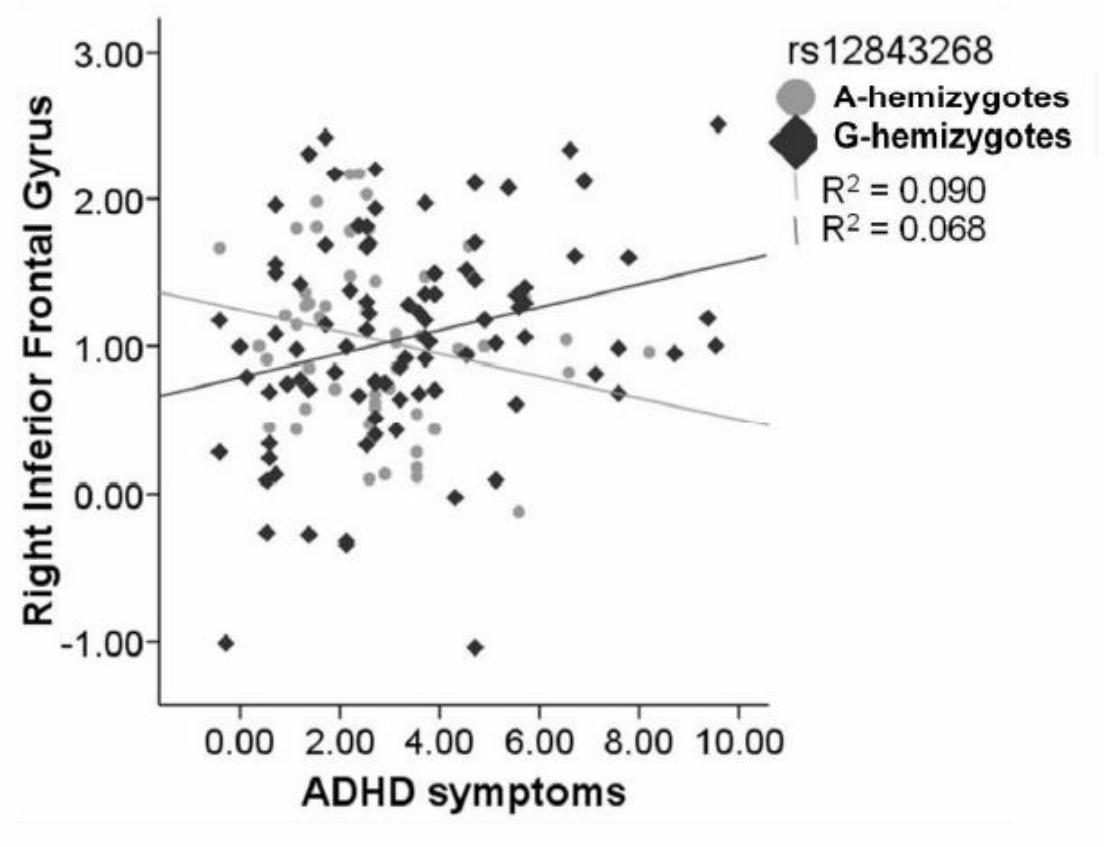
6.4.4.1 Association between MAOA rs12843268 and right IFG activation in boys

We measured BOLD-response of the right IFG using the SST in 143 out of the 190 boys. We observed no significant effect of rs12843268 genotype on BOLD-response in the right IFG ($t = 0.02$, $p = 0.88$).

6.4.4.2 Effect of MAOA rs12843268 on the relationship between right IFG activation and ADHD symptoms in boys

We found a significant *MAOA* rs12843268 x right IFG interaction on ADHD symptoms ($t = 6.24$, $p = 0.014$). Upon stratification by genotype we found a positive correlation between right IFG BOLD-response and ADHD symptoms ($r = 0.26$, $p = 0.017$) amongst G hemizygotes, whereas in A hemizygotes we found a negative correlation between right IFG BOLD-response and ADHD symptoms *(*$r = -0.30$, $p = 0.049$) ***(Figure 26)***.

Figure 26. Correlation between right IFG activation and ADHD symptoms: Right IFG activation was positively correlated with ADHD symptoms in G hemizygotes (r = 0.26, p = 0.017). In A hemizygotes a negative correlation was found between right IFG activation and ADHD symptoms (r = -0.30, p = 0.049).



Without stratification by rs12843268 the neural responses in the right IFG accounted for 1.7% of the variance in ADHD symptoms whereas after stratification 6.8% of the variance in ADHD symptoms was accounted for in G hemizygotes and 9% of the variance in ADHD symptoms was accounted for in A hemizygotes.

Due to a mistake in the paradigm for collecting stop signal reaction time (SSRT), data were only available for 73 individuals out of 143 individuals **(*Table 13*)**. There was no significant difference between A hemizygotes and G hemizygotes in SSRT and no genotype differences in the number of successfully completed Stop

trials or Go trials (***Table 13*** and ***Table 14***). However, we found a negative correlation between SSRTs and right IFG BOLD-response (r = -0.28, p = 0.02), indicating that higher BOLD-response of the right IFG during successful inhibition trials was associated with lower SSRT. This association was significant in G hemizygotes (r = -0.36, p = 0.02) but not in A hemizygotes (r = -0.15, p = 0.44) **(*Table 14*)**.

Table 13: Performance on the SST – based on number of Stop success and Go success trials: Means and standard deviations are presented for the number of successful Stop trials and Go trials by genotype and full sample. Number of successful Go trials and successful Stop trials did not differ by genotypes.

	A (n=52)	***G (n=91)***	***Full sample (n=143)***	***Genotype differences***
Number of Stop Success Trials	35 ± 4.8	34 ± 6.8	34 ± 6.2	$t = 1.37$, $p = 0.17$
Number of Go Success Trials	377 ± 18.4	371 ± 31.2	373 ± 27.3	$t = 1.23$, $p = 0.22$

Table 14: Stop signal reaction times during the SST divided by rs12843268 genotype: Means and standard deviations are presented for SSRTs (ms) in full sample and by genotype. SSRT did not significantly differ between genotypes. SSRT was negatively correlated with right IFG BOLD-responses (data available for 73 out of 143 participants).

	A (n=31)	***G (n=42)***	***Full sample (n=73)***	***Genotype differences in RT***
SSRT (ms)	217.2 ± 35.2	220.3 ± 39.3	218.9 ± 37.4	$t = 0.18$, $p = 0.67$
Right IFG/SSRT correlation	$r = -0.15$, $p = 0.44$	$r = -0.36$, $p = 0.02$	$r = -0.28$, $p = 0.02$	

SSRT: Stop Signal Reaction Time, RT: Reaction Time, IFG: Inferior Frontal Gyrus

6.5 DISCUSSION

In a community-based sample we demonstrate that *MAOA* is associated with ADHD symptoms in boys, but not in girls. In boys we found that ADHD symptoms were correlated with distinct fronto-striatal activation patterns, depending on rs12843268 genotype.

6.5.1 THE EFFECT OF *MAOA* ON ADHD SYMPTOMS

We found a significant association between *MAOA* rs12843268 genotype and ADHD symptoms in boys, but not in girls. This is consistent with a previous study suggesting that *MAOA* is not associated with ADHD in girls (Manor, et al., 2002). It has been suggested that girls are able to compensate for a risk variant by having two X-chromosomes. Due to the X-linked nature of *MAOA*, it may affect ADHD symptoms less severely in girls than it does in boys. The fact that rs12843268 was not expressed in girls may explain why we did not find a significant effect of genotype. However, as stated in Chapter Four girls may not have displayed enough ADHD symptoms to detect an effect of *MAOA*. Based on these findings we investigated the effect of *MAOA* on brain activation patterns in boys.

6.5.2 THE EFFECT OF *MAOA* ON VS AND IFG ACTIVATION

As discussed in Chapter Five, *MAOA* was significantly associated with VS activation during reward anticipation in boys. Prior research suggests that *MAOA* affects brain activation (Meyer-Lindenberg, et al., 2006) during inhibitory control. This study was not able to identify a significant effect of *MAOA* and right IFG during successful inhibitory control. However, our study investigated a different region than Meyer-Lindenberg and colleagues who particularly investigated the effect of *MAOA* on anterior cingulate activation. We also targeted a different genetic variant (i.e.

rs12843268) than that presented in the study by Meyer-Lindenberg (i.e. the *MAOA*-VNTR).

6.5.3 *MAOA* STRATIFICATION

In boys, we tested whether ADHD symptoms were correlated with distinct fronto-striatal activation patterns, depending on rs12843268 genotype. We found that in A hemizygotes, who express lower levels of MAOA, ADHD symptoms are associated with lower VS BOLD-response indicating lower reward-related activity, as well as reduced inhibition as measured by right IFG BOLD-response. This may suggest that ADHD symptoms in this group arise from a blunted reward response coupled with lower prefrontal inhibitory control, as postulated by the reward deficiency syndrome (RDS) hypothesis (Blum, Cull, et al., 1996).

Conversely, in G hemizygotes who express higher levels of MAOA, ADHD symptoms were associated with increased right IFG BOLD-response in the presence of increased VS BOLD-response. The observed negative correlation of SSRT and right IFG BOLD-response in G hemizygotes suggest that higher VS BOLD-responses alone may not be a risk factor for ADHD symptoms in this group, but needs to be considered together with the requirement for larger frontal recruitment for optimal task performance (Schulz, et al., 2004). Based on previous studies which suggest that *MAOA* may affect the connectivity between prefrontal and subcortical regions (Buckholtz, et al., 2008) it is interesting that G hemizygotes, who show a greater level of ADHD symptoms also show a correlation between right IFG activation and SSRT. Previous research suggest that in cases where adolescents perform at adult levels on the SST, i.e. show equal levels of successful inhibition as adults, they show higher activation of the inferior frontal cortex as a means of compensating for the lack of connectivity between prefrontal and subcortical regions (Stevens, et al., 2007). Based

on these findings it is possible that the G hemizygotes of our study show increased right IFG activation in order to compensate for reduced connectivity.

Theories based on neuroimaging studies suggest two alternative mechanisms underlying ADHD symptoms: the impulsivity hypothesis suggests that insufficient inhibitory control underlies the disorder; the RDS hypothesis proposes that impulsive behaviours compensate for blunted sensitivity of the reward system (Bechara, 2005; Comings & Blum, 2000; Hommer, et al., 2011). Our results suggest that both mechanisms contribute to ADHD symptoms, depending on MAOA genotype. The *MAOA*-stratification of neural mechanisms underlying ADHD symptoms may contribute to the resolution of seemingly contradictory findings in the ADHD literature, which report both over- or under-activation of the inhibitory control network (Dickstein, et al., 2006; Ma, et al., 2011; Pliszka, et al., 2006; Rubia, et al., 2005; Schulz, et al., 2004).

6.5.4 *MAOA* EXPRESSION

In order to understand how rs12843268 affects BOLD-response in our sample we investigated the expression levels of MAOA in A hemizygotes and G hemizygotes. We find allele-specific gene expression differences in peripheral blood with G hemizygotes showing a 6-fold increase in MAOA expression, compared to A hemizygotes. This suggests that G hemizygous boys have higher *MAOA* mRNA levels, which might result in increased degradation of monoamines and lower baseline levels of serotonin, dopamine and noradrenaline.

Reduced levels of serotonin are known to enhance premature responding and are associated with higher impulsiveness (Robbins, 2010). Accordingly, G hemizygous boys showed a greater level of ADHD symptoms than A hemizygous boys. In G hemizygotes we found an association of increased right IFG BOLD-

response and high ADHD symptoms as well as a negative correlation between right IFG BOLD-response and shorter SSRTs. These results might suggest a requirement for higher brain activity in the key inhibitory region to achieve similar synaptic serotonin concentrations in G hemizygotes as compared to A hemizygotes, and to inhibit inappropriate responses in the SST in order to obtain similar behavioural results (Pliszka, et al., 2006; Schulz, et al., 2004). Lower MAOA levels in A hemizygotes might result in increased baseline levels of monoamines in the VS relative to G hemizygotes. As the motivational salience of a reward stimulus depends on the relative increase in dopamine (Samaha & Robinson, 2005), as opposed to the absolute level, an increased baseline might result in a smaller relative increase due to a ceiling effect in dopamine response.

6.5.5 IMPLICATIONS

Our results indicate that stratification of neuroimaging phenotypes by *MAOA* genotype notably increases the amount of variance explained. For example, BOLD-response during reward anticipation in the right VS of A hemizygotes accounts for 8.4% of the variance in ADHD symptoms. This contrasts with 2.2% of the variance accounted for by genotype on ADHD symptoms and 2.6% of the variance accounted for by right VS BOLD-response on ADHD symptoms, when both genotypes are considered jointly. In the case of right IFG BOLD-response we found that associations only became apparent upon stratification by *MAOA* genotype. This might explain recent results (Carmona, et al., 2011) which, in the absence of genetic analyses, failed to identify an association between ADHD and IFG BOLD-response during inhibition trials. However, our findings also suggest a sizeable proportion of unexplained variance, which can probably be accounted for by the influence of multiple genes as well as additional brain functions underlying ADHD symptoms.

6.5.6 LIMITATIONS

While ADHD symptoms are associated with ADHD status, measured by the SDQ, no 'probable' cases, and only 28 'possible' cases of ADHD were identified in our sample. Furthermore, the mean number of ADHD symptoms in our population-based sample is approximately 50% below the threshold for clinical ADHD (3.1 vs. 5). While this does not affect the interpretation of the association observed between ADHD symptoms and neurobiological functions, it indicates the normative character of our data, and the need for validation in ADHD patients to fully assess their clinical applicability.

It should be noted that in the use of the SST, this study investigated the contrast 'stop success vs. go success'. While this contrast controls for effects of motor planning and execution it is includes brain activation patterns that may be due to an oddball design, due to the fact that stop trials occur in 17% of the trials. Therefore we cannot exclude the possibility that the activation patterns observed were partly due to an oddball effect. However, this study targeted activation in the right IFG specifically, which is known to play an important role in response inhibition. This was supported by the correlation between right IFG activation and SSRTs. An alternative approach would have been to investigate the 'stop success vs. stop failure' contrast. However, this would not have allowed us to control for effects of motor planning and execution.

6.5.7 CONCLUSIONS

Through stratification of ADHD symptoms by *MAOA* genotype we identified two distinct fronto-striatal mechanisms that determine the manifestation of ADHD symptoms in adolescent boys; one of blunted reward and inhibitory control and another characterised by increased reward processing coupled with enhanced efforts

to recruit the top down frontal inhibitory system. Apart from its mechanistic interest, our discovery is of potential clinical relevance as it may provide the basis for a development of genetic stratification markers to predict therapeutic response to pharmacological interventions in ADHD.

7 CHAPTER SEVEN:

CONCLUSIONS

7.1 OVERVIEW OF FINDINGS

This thesis presented analyses of behavioural, neuroimaging and genetic data from a large sample of adolescents. We investigated the functioning of the reward system in adolescence and its relationship to novelty seeking and ADHD symptoms. We also explored the relationship between *MAOA* genotype and brain activation patterns during reward processing and inhibitory control.

In a recent review, Munoz et al. (2009) highlighted the importance of large-scale imaging genetic studies to examine the relationship between behaviour, neurological function and genetics in adequately powered samples (Munoz, Hyde, & Hariri, 2009). The IMAGEN study is the largest available adolescent imaging genetic dataset to date. This thesis used the IMAGEN dataset to investigate causes and effects of deficient reward processing (Schumann, et al., 2010). We characterised reward processing in the largest neuroimaging sample to date (Chapter Three) and identified gender differences in the reward system of adolescents (Chapter Four). The results of Chapter Three are in line with previous research on reward processing in adolescents and adults while the results of Chapter Four present novel gender differences in reward processing. Chapter Four also replicated the expected relationship between VS activation and ADHD symptoms, but the expected association is displayed only in boys. We also present novel associations between *MAOA* genotype and brain activation patterns during both reward processing and inhibitory control (Chapter Five and Chapter Six).

7.1.1 PRINCIPAL FINDINGS

In ***Table 15*** the original study objectives and hypotheses are restated with a brief summary of the relevant results and an indication of whether each hypothesis was supported or not. The following section will provide a more comprehensive description of the main results for each chapter. Of the 17 hypotheses investigated in this thesis, 12 were fully supported by the evidence obtained, 2 were partially supported and 3 were not supported.

Table 15: Summary of findings in relation to original objectives and hypotheses

Hypotheses and Objectives	***Supported?***	***Specific Results***
Based on prior literature we hypothesised that adolescents would activate a similar reward system as adults	Partially	✓ Random effects analyses of the 'anticipation high win vs. no win' contrast revealed BOLD-responses extending from the striatum to prefrontal and middle frontal cortex as well as to the parietal and occipital lobes. This is in accordance with prior literature of the reward system in adults ✓ Random effects analyses of the 'feedback high win vs. no win' contrast revealed BOLD-responses in the anterior and posterior cingulate gyrus, medial OFC and parietal lobe × Contrary to previous studies of reward processing, we did not identify a significant BOLD-response in the VS during the reward feedback phase
The activation of the VS is higher during reward anticipation than reward feedback (both measured during high win trials)	Yes	✓ The VS was significantly activated during reward anticipation, but not during reward feedback
The activation of the OFC is higher during reward feedback than reward anticipation	Partially	✓ The medial OFC showed significantly enhanced activation during reward feedback, whereas the middle OFC showed significantly higher activation during reward anticipation
There are significant gender differences in VS activation during reward anticipation	Yes	✓ Boys show significantly higher activation of the VS during reward anticipation relative to girls
There are significant gender differences in VS activation during reward feedback	Yes	✓ Boys show significantly higher activation of the VS during reward feedback relative to girls

Hypotheses and Objectives	*Supported?*	*Specific Results*
There are significant gender differences during whole brain analyses of both reward anticipation and reward feedback	Yes	✓ Boys show significantly higher activation of a number of regions relative to girls. During reward anticipation these include the caudate, precentral gyrus and superior frontal cortex. During reward feedback boys show greater activation of caudate, thalamus and cerebellum relative to girls
There are gender differences in the relationship between ADHD symptoms and VS activation measured during reward anticipation and reward feedback	Yes	✓ Boys show the expected negative correlation between ADHD symptoms and VS activation during reward anticipation ✓ Girls do not show the expected negative correlation between ADHD symptoms and VS activation × There is no significant relationship between ADHD symptoms and VS activation during reward feedback in boys or girls
There is a significant correlation between novelty seeking and VS activation during reward feedback	No	× There is no significant correlation between novelty seeking scores and VS activation during reward anticipation, in the full sample, in boys or in girls
MAOA rs12843268 genotype affects novelty seeking and impulsivity	No	× *MAOA* rs12843268 did not affect novelty seeking measured by the TCI in our sample
MAOA rs12843268 genotype affects VS activation differently in boys compared to girls during reward anticipation	Yes	✓ We found a significant association between *MAOA* rs12843268 genotype and VS activation in boys, but not in girls ✓ A hemizygous boys showed a significantly lower activation of the VS during reward anticipation relative to G hemizygous boys

Hypotheses and Objectives	*Supported?*	*Specific Results*
MAOA rs12843268 genotype stratifies the relationship between VS activation and novelty seeking	Yes	✓A hemizygous boys show a significant correlation between novelty seeking and VS activation during reward anticipation ✓G hemizygous boys do not show a significant correlation between novelty seeking and VS activation during reward anticipation
There are significant differences in the expression levels of the two alleles of *MAOA* rs12843268	Yes	✓ We found significant expression differences between A hemizygous and G hemizygous boys ✓ G hemizygous boys showed significantly higher expression levels than A hemizygous boys ✓ We found no significant differences in expression levels amongst girls
MAOA rs12843268 genotype affects ADHD symptoms	Yes	✓ *MAOA* rs12843268 significantly affects ADHD symptoms in boys ✓ G hemizygous boys show significantly more symptoms of ADHD compared to A hemizygous boys
ADHD symptoms are correlated with VS activation during reward anticipation	Yes	✓ We found a significant negative correlation between VS activation and ADHD symptoms in the full sample of boys
ADHD symptoms are correlated with right IFG activation during response inhibition	No	× We did not find a significant correlation between ADHD symptoms and right IFG activation during response inhibition in the full sample of boys

Hypotheses and Objectives	*Supported?*	*Specific Results*
MAOA rs12843268 genotype stratifies the relationship between ADHD symptoms and VS activation during reward anticipation in boys	Yes	✓ A hemizygous boys were driving the negative correlation between ADHD symptoms and VS activation found in the full sample ✓The correlation between ADHD symptoms and VS activation was not significant in G hemizygous boys
MAOA rs12843268 genotype stratifies the relationship between ADHD symptoms and right IFG activation during response inhibition in boys	Yes	✓ A hemizygous boys showed a negative correlation between ADHD symptoms and right IFG activation during response inhibition ✓ G hemizygous boys showed a positive correlation between ADHD symptoms and right IFG activation during response inhibition

7.1.2 CHAPTER THREE

In this thesis, neuroimaging analyses of reward processing were based on two contrasts of the MID task: the 'anticipation high win vs. anticipation no win' contrast and the 'feedback high win vs. feedback no win' contrast. By investigating activation patterns during the high win vs. no win contrasts, rather than the high win vs. small win contrasts, we were able to capture as much as possible of the signal associated with reward processing. Secondly, we chose the high win vs. no win contrasts, as opposed to high win vs. baseline, in order to minimise the variance related to non-reward processes, such as visual processing.

The results from Chapter Three show that adolescents activate similar brain regions during reward processing as previously shown in adults (Knutson, Adams, et al., 2001; Knutson, et al., 2000; Knutson & Cooper, 2005; Knutson, Fong, et al., 2001). Our results suggest that the VS is significantly activated during reward anticipation. The random effects analysis of reward anticipation also revealed widespread activation across a number of other regions such as the dorsal striatum, supplementary motor area, the cingulate gyrus and the OFC, which have been implicated as reward-regions in previous neuroimaging studies (Knutson, Adams, et al., 2001; Liu, et al., 2011).

The results from this study suggest that the importance of the VS during processing of reward feedback is less pronounced than suggested by prior research. Previous literature suggests that after an individual has learned the association between a cue (an unconditioned stimulus) and a reward, dopaminergic activation shifts from the unconditioned stimulus (i.e. the feedback-phase in the MID task) to the conditioned stimulus indicating that a reward can be expected (i.e. the anticipation phase during the MID task) (Schultz, et al., 1997). Our participants had been

familiarised with the MID task prior to the scanning session and their expectation to receive a reward was high following the display of a cue (in 66% of the trials the participant received a reward). Thus, it seems possible that amongst our participants the VS activation has shifted from the reward feedback phase to the reward anticipation phase. In prior studies the expectations to receive a reward based on the unconditioned stimulus may not have been so high. This may for example, be the result of the MID task being composed of both reward and punishment trials (Knutson, Adams, et al., 2001; Knutson, et al., 2000; Knutson, Fong, et al., 2001) which may increase the uncertainty of receiving a reward during a particular task. It is also possible that our results are due to the younger age of our participants in contrast to participants tested in previous studies.

A whole brain analysis suggested that the cingulate cortex and OFC were significantly activated during reward feedback. These regions are frequently associated with monitoring of rewards and planning of future actions based on attained rewards. In accordance with the literature our results suggest that the OFC plays an important role in reward processing during both phases of reward processing. During the reward anticipation phase the activation in the OFC is in the middle OFC, while during reward feedback the activations are centred in the medial OFC. The medial OFC is related to the monitoring, learning and memory of the reward value of reinforcers, whereas the middle OFC may play a role in response inhibition and the evaluation of losses (Kringelbach, 2005; Sescousse, et al., 2010).

7.1.3 CHAPTER FOUR

This chapter explored gender differences in reward processing during both reward anticipation and reward feedback. The results revealed that adolescent boys show significantly higher activation of several regions, including the VS during both phases of reward processing. During reward anticipation, boys also showed significantly higher activation of a number of other regions previously implicated in the reward network, including the cingulate cortex, caudate and superior frontal gyrus. The cingulate cortex is said to play an important role in evaluating the rewards and losses associated with errors (Bush et al., 2002). During the feedback phase boys showed significantly higher activation of traditional reward regions such as the caudate and thalamus, but also of motor areas such as the cerebellum and postcentral gyrus. The inverse analyses (girls > boys) did not yield any significant activations. The results indicate that the reward system of adolescent boys is significantly more active than that of adolescent girls in response to the MID task.

Previous studies have also identified gender differences in a number of reward-related disorders, such as ADHD (Arnold, 1996; Lentini, et al., 2012; Savic, 2010). Previous functional MRI studies of ADHD have mainly investigated brain activation patterns in boys (Paloyelis, et al., 2012; Scheres, et al., 2007b; Stoy, et al., 2011; Strohle, et al., 2008). We aimed to determine whether the relationship between ADHD symptoms and VS activation, measured during reward anticipation and reward feedback, is also found in girls. The results suggested that the significant negative correlation between ADHD symptoms and VS activation during reward anticipation was specific to boys. During reward feedback a trend was found between ADHD symptom-count and VS activation in boys. This suggests that reward-related

VS activation may act as a vulnerability factor for ADHD symptoms in boys, but be of lesser importance in girls.

This is one of the first studies that investigates the correlation between a dimensional measure of ADHD symptoms and neural activation. Previous research suggests that treating ADHD dimensionally rather than categorically increases sensitivity of correlations between measures, such as the Tower of Hanoi or the Continuous Performance Task, and ADHD symptoms relative to a categorical approach to analysis. Unfortunately we were unable to test the association between neural measures and a categorical variable of ADHD due to a very small number of ADHD patients in our community sample.

It should be noted that this study may be limited by the significantly lower level of ADHD symptoms amongst girls relative to boys in our sample. The symptom-count of the girls may not have been high enough to identify a relationship between VS activation and ADHD symptoms. It would be interesting to also investigate the association between VS activation and ADHD diagnosis. In our sample no participant met the criteria for ADHD diagnosis.

7.1.4 CHAPTER FIVE

Gender differences in reward-related behaviour are often attributed to genes on the X-chromosome. We found that a particular SNP, rs12843268, within the X-linked *MAOA* gene is associated with VS activation during reward anticipation in boys and girls.

The results suggested that *MAOA* affects VS activation in boys, but not in girls. However, it is worth noting that only very few girls in our sample were homozygous for the minor allele (A) of rs12843268 (n=16). Thus, the sample may not be large enough to investigate the effect of the minor allele in girls. However, it is

also interesting that rs12843268 was not expressed in girls, whereas the polymorphism was expressed in boys. The lack of expression of this SNP amongst girls may be the cause of the non-significant genotype differences in VS activation.

It is suggested that *MAOA* represents one genetic mechanism underlying novelty seeking in boys. Prior literature has associated *MAOA* genotype with novelty seeking measured by the TCI (Shiraishi, et al., 2006). However, in our study, we were unable to replicate this association. This may be due to the fact that Shiraishi and colleagues associated the *MAOA*-VNTR, rather than particular SNPs, with measures of the TCI. Unfortunately, the IMAGEN study does not have access to VNTR-data. The participants of Shiraishi et al.'s study were also substantially older than our sample (mean age: 29.9 compared to 14.4 in our sample).

Whereas we were unable to replicate prior work suggesting that *MAOA* is associated with novelty seeking and impulsivity we revealed that A hemizygous boys showed a negative correlation between novelty seeking and VS activation. VS activation has previously been correlated with novelty seeking and impulsivity (Wittmann, et al., 2008), suggesting that *MAOA* genotype may the relationship between novelty seeking and VS activation in boys.

7.1.5 Chapter Six

This chapter aimed to investigate whether *MAOA* was associated with ADHD symptoms and whether *MAOA* would stratify the relationship between ADHD symptoms and VS activation. As suggested by previous literature a significant association between *MAOA* genotype and ADHD symptoms was found in boys, but not in girls. *MAOA* was also associated with VS activation in boys, but did not have an effect on IFG activation measured during inhibitory control trials.

We also found that neural mechanisms of ADHD were stratified by *MAOA* genotype. The results suggested that both VS activation, measured during reward anticipation, and IFG activation, measured during inhibitory control, contribute to ADHD symptoms in adolescent boys.

We demonstrated an association of ADHD symptoms with distinct BOLD-responses depending on *MAOA* genotype. In A hemizygous boys of SNP rs12843268, who express lower levels of MAOA, ADHD symptoms were associated with lower VS BOLD-response and lower right IFG BOLD-response. In G hemizygous boys, ADHD symptoms were associated with increased right IFG BOLD-response during successful response inhibition and increased VS BOLD-response during reward anticipation. Thus, depending on *MAOA* genotype, ADHD symptoms in adolescent boys are associated with either reward deficiency or insufficient response inhibition.

7.2 OVERARCHING DISCUSSION

7.2.1 REWARD DEFICIENCY HYPOTHESIS VS. IMPULSIVITY HYPOTHESIS

This thesis confirms that the human reward system is of a complex nature. Previously it has been suggested that disordered behaviours result from over- or under-activation of the reward system. Below, we discuss which of our findings support the impulsivity hypothesis (overactivation of reward system) and which support the reward deficiency hypothesis (underactivation of the reward system).

Table 16 shows how the results of this thesis conform to the reward deficiency hypothesis and impulsivity hypothesis.

Table 16: Summary of findings that conforms to reward deficiency hypothesis and/or the impulsivity hypothesis

Prediction	***Results Supporting RDH***	***Results Supporting Imp. Hypothesis***	***Results***
Gender: ▪ Literature suggests that adolescent boys are more impulsive and show more externalising disorders than girls – if this is reflected in VS activation, we would expect the following: ▪ If boys show reduced activation of the VS relative to girls the results support the RDH ▪ If boys show increased activation of the VS relative to girls the results support the Impulsivity hypothesis	✗	✓	▪ We found that boys show higher activation of the VS relative to girls during both reward anticipation and reward feedback
Novelty Seeking: ▪ Based on literature VS activation is correlated with novelty seeking and impulsivity ▪ A negative correlation between VS activation and novelty seeking/impulsivity supports the RDH ▪ A positive correlation between VS activation and novelty seeking/impulsivity supports the Impulsivity hypothesis	✓	✗	▪ In the full sample we found no correlation between VS activation and novelty seeking ▪ We found no correlation between VS activation and novelty seeking in boys or girls ▪ When boys were divided by *MAOA* genotype we found a correlation between VS activation and novelty seeking in A hemizygotes

Prediction	*Results Supporting RDH*	*Results Supporting Imp. Hypothesis*	*Results*
ADHD symptoms: ▪ Literature suggests that ADHD patients show reduced VS activation relative to controls. However, studies of ADHD tend to investigate only one phase of reward processing ▪ A negative correlation between ADHD symptoms and VS activation during either phase of MID support the RDH ▪ A positive correlation between ADHD symptoms and VS activation during either phase of MID supports the Impulsivity hypothesis	✓	×	▪ We found a negative correlation between ADHD symptoms and VS activation during reward anticipation in the full sample ▪ When dividing the sample by gender we found that this correlation was driven by the boys ▪ The correlation between ADHD symptoms and VS activation was not significant in girls

Based on prior literature we predicted a negative correlation between ADHD symptoms and VS activation during reward anticipation in boys. However, this finding is not easily consolidated with the finding that boys show elevated VS activation during reward anticipation and also a higher level of ADHD symptoms relative to girls.

It is possible that the discrepant results reflect that adolescent boys and girls differ in their baseline VS activation levels so that boys who do not show elevated VS activation during reward anticipation are more likely to show ADHD symptoms. Another explanation may be that VS activation during reward anticipation does not affect ADHD symptoms alone, but contributes to ADHD symptoms in combination with VS activation during reward feedback. The results of Chapter Four suggested that boys show significantly higher activation of the VS relative to girls during the reward feedback phase. This result is consolidated with the fact that boys also show a trend toward a positive correlation between VS activation and ADHD symptoms during the reward feedback phase. Few previous studies report data on the relationship between ADHD and brain activation patterns during reward feedback (Strohle, et al., 2008). The findings of this thesis suggest the importance of investigating activation patterns during both phases of reward processing in order to better understand the reward-related deficiencies that underlie externalising disorders.

The imaging genetic findings of this thesis (Chapter Five and Chapter Six) suggested that novelty seeking and ADHD symptoms may result from different processes in different individuals based on their *MAOA* genotype. In Chapter Five we found a negative correlation between VS activation and novelty seeking in boys, but only after *MAOA* stratification. These results are broadly in line with the reward deficiency hypothesis. Importantly, no significant correlation was found between the

TCI novelty seeking scale and ADHD symptoms, suggesting that these two measures do not measure the same thing. The results of Chapter Six are of particular interest as we demonstrate an association of ADHD symptoms with distinct BOLD-responses depending on *MAOA* genotype. In boys who were G hemizygous for rs12843268, ADHD symptoms were associated with increased right IFG BOLD-response during SST in the presence of increased VS BOLD-response during MID. This pattern of activation suggests that G hemizygous boys conform to the impulsivity hypothesis. In A hemizygotes on the other hand ADHD symptoms negatively correlated with both VS BOLD-response during MID and right inferior frontal gyrus (IFG) BOLD-response during the SST. The pattern of activation amongst A hemizygotes appears to conform to the reward deficiency hypothesis. Thus, the results of this study suggest that individuals may display brain activation patterns in line with either the reward deficiency hypothesis or the impulsivity hypothesis depending on reward-related genotypes, such as *MAOA*.

In conclusion, neither hypothesis fully explains all relations between reward-related brain activation and behaviour. We suggest that future research which aims to investigate the reward deficiency and/or impulsivity hypotheses investigate reward anticipation and reward feedback separately in order to determine whether over- and/or underactivation of the VS occurs during one or both stages. We also suggest that results are stratified by gender in order to determine whether the reward system of boys and girls show fundamentally different activation patterns as shown in this thesis. Finally, we suggest that genetic stratification of dopaminergic genes may facilitate our understanding of the developing reward system.

7.2.2 GENDER DIFFERENCES

Gender differences in brain activation patterns are rarely investigated. Functional MRI studies that recruit both males and females usually lack the power to divide the sample by gender. Other studies recruit only males or only females depending on the phenotype investigated. Thus, we know little about gender specific brain activation patterns underlying psychiatric disorders.

Many reward-related psychiatric disorders, such as ADHD, are more prevalent in males than females. ADHD patients frequently show reward deficiencies, particularly in VS activation measured during reward anticipation. Due to the task-design of prior studies it is unclear whether these deficiencies are gender-specific. The results of Chapter Four showed that deficient reward processing is specific to boys in our community sample of adolescents. However, in order to reach conclusive results on the relationship between ADHD symptoms and VS activation in girls our results need to be replicated in a sample of girls with a higher level of ADHD symptoms.

Prior research suggests that genes on the X-chromosome are responsible for behavioural differences between the genders. *MAOA* is one X-linked gene thought to mediate gender differences in impulsive behaviour.

The results of Chapter Five suggest that *MAOA* has an effect on VS activation in boys, but not in girls. G hemizygous boys showed significantly higher activation of the VS during reward anticipation relative to A hemizygous boys. However, *MAOA* genotype did not significantly affect VS activation amongst girls.

Considering these results it is interesting that rs12843268 is expressed in boys, with G hemizygotes showing significantly higher expression relative to A hemizgotes. We found no significant differences between the allele groups in girls,

which may explain why rs12843268 does not appear to have a significant effect on VS activation in girls. Several reasons may explain why *MAOA* is expressed in boys, but not in girls. Firstly, genes on one of the X-chromosomes carried by girls are randomly inactivated by methylation. Thus, we do not know which allele is expressed in girls who are heterozygous for *MAOA*. Secondly, research suggests that sex hormones such as estrogen and testosterone may affect the expression of MAOA. Considering that sex hormones are particularly active in adolescence it is possible that this affects the expression levels of the gene and, thus, behaviour. Further translational investigations are necessary to fully understand the effect of hormones on MAOA expression and behaviour.

7.3 METHODOLOGICAL ISSUES

The IMAGEN study has many strengths including its large sample size and multimodal nature; nevertheless it is also subject to various methodological limitations which must be considered when interpreting the results. Discussions of the methodological issues pertaining to the different aspects of this thesis are provided at the end of each analysis section. The main issues will be revisited here:

Community sample. The IMAGEN study is based on a community sample. Thus, we are unable to investigate differences between cases and controls. Considering that the participants of imaging are rather young (14 years), many psychiatric disorder may not yet have developed.

Centre effects. The data presented in this thesis were collected at 8 centres in Europe (London, Nottingham, Dublin, Berlin, Hamburg, Mannheim, Paris and Dresden). The activation patterns of the MID task showed substantial centre-differences in the whole

brain analyses. Thus, all analyses were covaried for centre effects. However, we still do not fully understand the reasons behind these effects. Some centre differences may result from the fact that data is collected on three different types of scanners, whereas other centre effects may be the result of differences in task-administration.

Puberty development. Dopaminergic affinity, which is known to underlie reward processing, is affected by pubertal hormones. IMAGEN measures pubertal development using the Puberty Development Scale (PDS). As expected the PDS suggest that boys and girls within our 14-year old cohort show very different patterns of puberty development. Whereas the girls score at the late pubertal or postpubertal end on the PDS, the boys are in the prepubertal or early pubertal stages. Thus, we are unable to determine the effect of pubertal development in reward-related gender differences.

MID contrasts. Based on the IMAGEN dataset, 44 contrasts of the MID task have been calculated. This thesis used only two of these contrasts, the 'anticipation high win vs. anticipation no win' and the 'feedback high win vs. feedback no win'. We chose the high win vs. no win contrasts, as opposed to high win vs. baseline, in order to minimise the variance related to non-reward related processes. These contrasts capture as much as possible of the signal associated with reward processing (i.e. by investigating activation patterns during the high win vs. no win contrasts, rather than the high win vs. low win). However, many more aspects of reward processing need to be investigated, such as how humans respond to varying magnitude of reward and the omission of an expected reward.

Large imaging datasets: The use of large data sets comes with potential challenges in terms of brain coverage in second level analyses. This is due to a small number of voxels being excluded in many participants due to lacking activation (which may for example be the case when the top of the brain is outside the magnetic scanning field). Since these voxels are not necessarily overlapping across participants, larger datasets may result in a larger number of voxels being excluded from analyses. This challenge is prominent in the random effects analyses displayed in chapter 3. However, chapters 4, 5 and 6, which mainly investigate ROIs in subcortical regions and the frontal cortex were not affected by this predicament.

7.4 IMPLICATIONS FOR FUTURE RESEARCH

The findings presented in this thesis have not only broadened our understanding of reward processing, but it also has implications for future research.

Firstly, we found substantial gender differences during both phases of reward processing. In order to reduce confounding effects we suggest that functional MRI studies of reward processing investigate reward processing in boys and girls separately or covary for gender differences in their analyses.

Secondly, many studies of reward processing are based on data from either the reward anticipation or the reward feedback phase. However, based on prior work by Schultz et al., but also by data presented here, the two phases of reward processing are related; i.e. increased activation during reward anticipation will result in reduced activation during reward feedback. In order to fully understand the relationship between reward processing and behavioural traits and disorders, data from both phases should be reported. Furthermore, descriptions of the reward system as over- or underactivation appear largely unhelpful as a negative correlation may be found between VS activation and behaviour during one reward anticipation whereas the

inverse may be found during reward feedback (as shown in Chapter Four). This implies that many of the papers that present relationships between VS activation and traits or disorder status during reward anticipation may not portray enough of the picture to say that the reward system is over- or underactivated.

Thirdly, prior research suggests that ADHD patients show significantly lower activation of the VS than healthy controls. Our data suggest that there is also a negative correlation between ADHD symptoms and VS activation in a healthy adolescent population.

Finally, dopaminergic genes are frequently discussed in relationship with reward processing. Based on results from Chapter Five and Chapter Six we showed that *MAOA*, which is known to encode the MAOA enzyme which degrades dopamine and serotonin in the brain, has an effect on VS activation in boys only. In a time of increasing efforts to develop personalised psychopharmacological therapies this may be an important finding if reliably replicated across ages.

7.5 DIRECTIONS FOR FUTURE RESEARCH

In consideration of the methodological limitations of the present series of studies and the remaining gaps in our knowledge concerning the factors which influence the development of reward processing, a number of suggestions are proposed regarding future research.

1. Study patients rather than a population based cohort. This thesis focused on population based data in healthy individuals. No individuals in this dataset reached diagnostic criteria for ADHD. Future work would need to investigate whether the findings of Chapter Four and Chapter Six are true in a clinical population of ADHD patients.

2. Investigate prediction error. BOLD-responses during reward anticipation and reward feedback are related to each other. Schultz et al. referred to this relationship as the prediction error, i.e. the difference in brain activation observed during predicted and brain activation during experienced reward. In order to understand the relationship between reward processing and ADHD, novelty seeking or impulsive behaviours it may not be sufficient to investigate each phase separately. Instead, we would need to investigate the relationship between the two phases, or the so-called prediction error.

3. Follow-up of behavioural data. Behavioural data of ADHD symptoms, novelty seeking and impulsivity were collected again at age 16. Using this data we can test the predictive value of genetics and neuroimaging phenotypes for the development of disorders and behaviour.

4. Follow-up of neuroimaging data. The literature suggests that the way humans process rewards change across development. Functional MRI data of the IMAGEN sample are planned to be collected a second time at age 18. This data will tell us whether reward processing will change with age and the directionality of such changes. The data will also be informative in investigations of whether the effects of gender and genotype on brain activation are found only during adolescence or whether they are stable throughout development.

5. Gene-gene interactions. This thesis investigated the effect of one SNP within the *MAOA* gene on ADHD symptoms, novelty seeking and impulsivity. However, *MAOA* is likely to interact with other dopaminergic genes in order to create deficits in reward processing, for example a recent study suggest that *MAOA* interact with *COMT* to predict intelligence in boys with ADHD (Qian et al. 2010).

6. Gene-environment interactions. *MAOA* is frequently investigated in interaction with environmental factors. Although not part of this thesis, stressful life events are measured in IMAGEN. In future studies we want to determine whether ADHD symptoms are affected by interactions between *MAOA* and stressful life events.

7. DNA Methylation. The expression of genes can be affected by DNA methylation. It is believed that DNA methylation is the result of environmental influences. Several studies have investigated the effect of *MAOA* DNA methylation on behaviour. DNA methylation of this gene is associated with schizophrenia, antisocial personality disorder, nicotine dependence and alcohol dependence (Chen et al. 2012; Philibert et al. 2011; Philibert et al. 2008). Further research is needed to determine whether these associations are mediated by the effect of *MAOA* on reward-related brain activation patterns.

8. Replication. The results of Chapter Five and Chapter Six need to be replicated in the full sample of IMAGEN.

7.6 FINAL CONCLUSIONS

This thesis relied upon the availability of a large multimodal imaging genetic dataset. The importance of the large dataset is revealed in the whole brain analyses of the reward system during reward anticipation and reward feedback, presented in Chapter Three. The results of the whole brain analyses correspond well with prior literature, but the consistency of the activation patterns across sample sizes and individuals increases the reliability of our findings and extend prior research of reward processing.

Previous literature suggests that patients with ADHD show reduced activation of the VS during reward anticipation. Our results reveal that in a healthy sample of adolescents this association takes the shape of a negative correlation between VS activation and ADHD symptoms. This negative correlation is driven by the boys in our sample. This suggests the importance of investigating gender differences in brain function, or if this is not possible due to small sample sizes, to covary for gender in analyses of the reward system.

Prior studies of the reward system are frequently based on small samples. Thus, few studies have had the opportunity to investigate gender differences. It is suggested that gender differences in reward processing may be particularly pertinent during the adolescent years when sexual development peaks. We found that reward processing differs between adolescent boys and girls and that these gender differences may be genetically mediated by *MAOA* genotype.

Following up on these findings, we investigated the relationship between *MAOA*, brain activation patterns and ADHD symptoms in boys. *MAOA* is shown to affect ADHD symptoms, but *MAOA* also affects VS activation. We found that based on *MAOA* genotype, adolescent boys may show a higher level of ADHD symptoms

either due to reduced VS activation or due to increased VS activation in combination with increased right IFG activation.

Replication of these findings is clearly required in other large samples using functional MRI data. In addition, the relationship and interplay between genetics, reward processing and behaviour needs to be explored longitudinally to enhance our understanding of the developing reward system and its effects on behaviour.

8 REFERENCES

Aichert, D. S., Wostmann, N. M., Costa, A., Macare, C., Wenig, J. R., Moller, H. J., et al. (2012). Associations between trait impulsivity and prepotent response inhibition. *Journal of Clinical and Experimental Neuropsychology, 34*(10), 1016-1032.

Alexander, G. E., Crutcher, M. D., & Delong, M. R. (1990). Basal Ganglia-Thalamocortical Circuits - Parallel Substrates for Motor, Oculomotor, Prefrontal and Limbic Functions. *Progress in Brain Research, 85*, 119-146.

American Psychiatric Association, A. (2000). *Diagnostic and statistical manual of mental disorders : DSM-IV-TR*. Washington, DC: American Psychiatric Association.

Arnett, J. (1992). Reckless Behavior in Adolescence - a Developmental Perspective. *Developmental Review, 12*(4), 339-373.

Arnold, L. E. (1996). Sex differences in ADHD: Conference summary. *Journal of Abnormal Child Psychology, 24*(5), 555-569.

Aron, A. R., Robbins, T. W., & Poldrack, R. A. (2004). Inhibition and the right inferior frontal cortex. *Trends Cogn Sci, 8*(4), 170-177.

Bechara, A. (2005). Decision making, impulse control and loss of willpower to resist drugs: a neurocognitive perspective. *Nature Neuroscience, 8*(11), 1458-1463.

Becker, J. B. (1999). Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacology Biochemistry and Behavior, 64*(4), 803-812.

Bjork, J. M., Knutson, B., Fong, G. W., Caggiano, D. M., Bennett, S. M., & Hommer, D. W. (2004). Incentive-elicited brain activation in adolescents: Similarities and differences from young adults. *Journal of Neuroscience, 24*(8), 1793-1802.

Blakemore, S. J., & Choudhury, S. (2006). Development of the adolescent brain: implications for executive function and social cognition. *Journal of Child Psychology and Psychiatry, 47*(3-4), 296-312.

Blum, K., Cull, J. G., Braverman, E. R., & Comings, D. E. (1996). Reward deficiency syndrome. *American Scientist, 84*(2), 132-145.

Blum, K., & Noble, E. P. (1990). Allelic Association of Human Dopamine-D2 Receptor Gene in Alcoholism - Reply. *Jama-Journal of the American Medical Association, 264*(14), 1808-1809.

Blum, K., Sheridan, P. J., Wood, R. C., Braverman, E. R., Chen, T. J. H., Cull, J. G., et al. (1996). The D-2 dopamine receptor gene as a determinant of reward deficiency syndrome. *Journal of the Royal Society of Medicine, 89*(7), 396-400.

Bodi, N., Keri, S., Nagy, H., Moustafa, A., Myers, C. E., Daw, N., et al. (2009). Reward-learning and the novelty-seeking personality: a between- and within-subjects study of the effects of dopamine agonists on young Parkinsons patients. *Brain, 132*, 2385-2395.

Breslau, J., Miller, E., Chung, W. J. J., & Schweitzer, J. B. (2011). Childhood and adolescent onset psychiatric disorders, substance use, and failure to graduate high school on time. *Journal of Psychiatric Research, 45*(3), 295-301.

Brookes, K., Xu, X., Chen, W., Zhou, K., Neale, B., Lowe, N., et al. (2006). The analysis of 51 genes in DSM-IV combined type attention deficit hyperactivity

disorder: association signals in DRD4, DAT1 and 16 other genes (vol 11, pg 934, 2006). *Molecular Psychiatry, 11*(12), 1139-1139.

Buckholtz, J. W., Callicott, J. H., Kolachana, B., Hariri, A. R., Goldberg, T. E., Genderson, M., et al. (2008). Genetic variation in MAOA modulates ventromedial prefrontal circuitry mediating individual differences in human personality. *Molecular Psychiatry, 13*(3), 313-324.

Bush, G., Vogt, B. A., Holmes, J., Dale, A. M., Greve, D., Jenike, M. A., et al. (2002). Dorsal anterior cingulate cortex: A role in reward-based decision making. *Proceedings of the National Academy of Sciences of the United States of America, 99*(1), 523-528.

Caldu, X., & Dreher, J. C. (2007). Hormonal and genetic influences on processing reward and social information. *Social Cognitive Neuroscience of Organizations, 1118*, 43-73.

Carmona, S., Hoekzema, E., Ramos-Quiroga, J. A., Richarte, V., Canals, C., Bosch, R., et al. (2011). Response inhibition and reward anticipation in medication-naive adults with attention-deficit/hyperactivity disorder: A within-subject case-control neuroimaging study. *Hum Brain Mapp*.

Casey, B. J., Getz, S., & Galvan, A. (2008). The adolescent brain. *Developmental Review, 28*(1), 62-77.

Chambers, R. A., Taylor, J. R., & Potenza, M. N. (2003). Developmental neurocircuitry of motivation in adolescence: A critical period of addiction vulnerability. *American Journal of Psychiatry, 160*(6), 1041-1052.

Cloninger, C. R., Bayon, C., & Svrakic, D. M. (1998). Measurement of temperament and character in mood disorders: a model of fundamental states as personality types. *Journal of Affective Disorders, 51*(1), 21-32.

Cloninger, C. R., Przybeck, T. R., & Svrakic, D. M. (1991). The Tridimensional Personality Questionnaire - United-States Normative Data. *Psychological Reports, 69*(3), 1047-1057.

Comings, D. E., & Blum, K. (2000). Reward deficiency syndrome: genetic aspects of behavioral disorders. *Cognition, Emotion and Autonomic Responses: The Integrative Role of the Prefrontal Cortex and Limbic Structures, 126*, 325-341.

Cook, E. H., Stein, M. A., Krasowski, M. D., Cox, N. J., Olkon, D. M., Kieffer, J. E., et al. (1995). Association of Attention-Deficit Disorder and the Dopamine Transporter Gene. *American Journal of Human Genetics, 56*(4), 993-998.

Corr, P. J. (2004). Reinforcement sensitivity theory and personality. *Neuroscience and Biobehavioral Reviews, 28*(3), 317-332.

Corr, P. J. (2008). The reinforcement sensitivity theory of personality and psychopathology. *International Journal of Psychophysiology, 69*(3), 151-152.

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nature Reviews Neuroscience, 3*(8), 655-666.

Dahl, R. E. (2004). Adolescent brain development: A period of vulnerabilities and opportunities - Keynote address. *Adolescent Brain Development: Vulnerabilities and Opportunities, 1021*, 1-22.

Das, M., Das Bhowmik, A., Sinha, S., Chattopadhyay, A., Chaudhuri, K., Singh, M., et al. (2006). MAOA promoter polymorphism and attention deficit hyperactivity disorder (ADHD) in Indian children. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics, 141B*(6), 637-642.

de la Rie, S. M., Duijsens, I. J., & Cloninger, C. R. (1998). Temperament, character, and personality disorders. *Journal of Personality Disorders, 12*(4), 362-372.

Delgado, M. R. (2007). Reward-related responses in the human striatum. *Reward and Decision Making in Corticobasal Ganglia Networks, 1104*, 70-88.

Dickstein, S. G., Bannon, K., Castellanos, F. X., & Milham, M. P. (2006). The neural correlates of attention deficit hyperactivity disorder: an ALE meta-analysis. *Journal of Child Psychology and Psychiatry, 47*(10), 1051-1062.

Domschke, K., Sheehan, K., Lowe, N., Kirley, A., Mullins, C., O'Sullivan, R., et al. (2005). Association analysis of the monoamine oxidase A and B genes with attention deficit hyperactivity disorder (ADHD) in an Irish sample: Preferential transmission of the MAO-A 941G allele to affected children. *American Journal of Medical Genetics - Neuropsychiatric Genetics, 134 B*(1), 110-114.

Dreher, J. C., Kohn, P., Kolachana, B., Weinberger, D. R., & Berman, K. F. (2009). Variation in dopamine genes influences responsivity of the human reward system. *Proc Natl Acad Sci U S A, 106*(2), 617-622.

Eisenberg, J., Mei-Tal, G., Steinberg, A., Tartakovsky, E., Zohar, A., Gritsenko, I., et al. (1999). Haplotype relative risk study of catechol-O-methyltransferase (COMT) and attention deficit hyperactivity disorder (ADHD): Association of the high-enzyme activity val allele with ADHD impulsive-hyperactive phenotype. *American Journal of Medical Genetics, 88*(5), 497-502.

Ernst, M., & Fudge, J. L. (2009). A developmental neurobiological model of motivated behavior: Anatomy, connectivity and ontogeny of the triadic nodes. *Neuroscience and Biobehavioral Reviews, 33*(3), 367-382.

Ernst, M., Nelson, E. E., Jazbec, S., McClure, E. B., Monk, C. S., Leibenluft, E., et al. (2005). Amygdala and nucleus accumbens in responses to receipt and omission of gains in adults and adolescents. *Neuroimage, 25*(4), 1279-1291.

Ernst, M., Nelson, E. E., McClure, E. B., Monk, C. S., Munson, S., Eshel, N., et al. (2004). Choice selection and reward anticipation: an fMRI study. *Neuropsychologia, 42*(12), 1585-1597.

Ernst, M., Pine, D. S., & Hardin, M. (2006). Triadic model of the neurobiology of motivated behavior in adolescence. *Psychological Medicine, 36*(3), 299-312.

Fairchild, G. (2011). The developmental psychopathology of motivation in adolescence. *Dev Cogn Neurosci, 1*(4), 414-429.

Faraone, S. V., & Doyle, A. E. (2001). The nature and heritability of attention-deficit/hyperactivity disorder. *Child and Adolescent Psychiatric Clinics of North America, 10*(2), 299-+.

Faraone, S. V., Perlis, R. H., Doyle, A. E., Smoller, J. W., Goralnick, J. J., Holmgren, M. A., et al. (2005). Molecular genetics of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 57*(11), 1313-1323.

Farrington, D. P. (1989). Self-Reported and Official Offending from Adolescence to Adulthood. *Cross-National Research in Self-Reported Crime and Delinquency, 50*, 399-423.

Forbes, E. E., Brown, S. M., Kimak, M., Ferrell, R. E., Manuck, S. B., & Hariri, A. R. (2009). Genetic variation in components of dopamine neurotransmission impacts ventral striatal reactivity associated with impulsivity. *Molecular Psychiatry, 14*(1), 60-70.

Friedman, N. P., & Miyake, A. (2004). The relations among inhibition and interference control functions: A latent-variable analysis. *Journal of Experimental Psychology-General, 133*(1), 101-135.

Galvan, A. (2010). Adolescent development of the reward system. *Frontiers in Human Neuroscience, 4*.

Galvan, A., Hare, T., Voss, H., Glover, G., & Casey, B. J. (2007). Risk-taking and the adolescent brain: who is at risk? *Developmental Science, 10*(2), F8-F14.

Galvan, A., Hare, T. A., Parra, C. E., Penn, J., Voss, H., Glover, G., et al. (2006). Earlier development of the accumbens relative to orbitofrontal cortex might underlie risk-taking behavior in adolescents. *Journal of Neuroscience, 26*(25), 6885-6892.

Gaub, M., & Carlson, C. L. (1997). Gender differences in ADHD: A meta-analysis and critical review (vol 36, pg 1036, 1997). *Journal of the American Academy of Child and Adolescent Psychiatry, 36*(12), 1783-1783.

Geier, C., & Luna, B. (2009). The maturation of incentive processing and cognitive control. *Pharmacology Biochemistry and Behavior, 93*(3), 212-221.

Geier, C. F., Terwilliger, R., Teslovich, T., Velanova, K., & Luna, B. (2010). Immaturities in Reward Processing and Its Influence on Inhibitory Control in Adolescence. *Cerebral Cortex, 20*(7), 1613-1629.

Giedd, J. N., Schmitt, J. E., & Neale, M. C. (2007). Structural brain magnetic resonance imaging of pediatric twins. *Human Brain Mapping, 28*(6), 474-481.

Gizer, I. R., Ficks, C., & Waldman, I. D. (2009). Candidate gene studies of ADHD: a meta-analytic review. *Human Genetics, 126*(1), 51-90.

Goodman, R. (1997). The Strengths and Difficulties Questionnaire: a research note. *J Child Psychol Psychiatry, 38*(5), 581-586.

Goodman, R. (2001). Psychometric properties of the strengths and difficulties questionnaire. *J Am Acad Child Adolesc Psychiatry, 40*(11), 1337-1345.

Gottesman, I. I., & Gould, T. D. (2003). The endophenotype concept in psychiatry: Etymology and strategic intentions. *American Journal of Psychiatry, 160*(4), 636-645.

Gu, X. S., Liu, X., Guise, K. G., Naidich, T. P., Hof, P. R., & Fan, J. (2010). Functional Dissociation of the Frontoinsular and Anterior Cingulate Cortices in Empathy for Pain. *Journal of Neuroscience, 30*(10), 3739-3744.

Guan, L., Wang, B., Chen, Y., Yang, L., Li, J., Qian, Q., et al. (2009). A high-density single-nucleotide polymorphism screen of 23 candidate genes in attention deficit hyperactivity disorder: Suggesting multiple susceptibility genes among Chinese Han population. *Molecular Psychiatry, 14*(5), 546-554.

Hahn, T., Heinzel, S., Dresler, T., Plichta, M. M., Renner, T. J., Markulin, F., et al. (2011). Association between reward-related activation in the ventral striatum and trait reward sensitivity is moderated by dopamine transporter genotype. *Hum Brain Mapp, 32*(10), 1557-1565.

Hampshire, A., Chamberlain, S. R., Monti, M. M., Duncan, J., & Owen, A. M. (2010). The role of the right inferior frontal gyrus: inhibition and attentional control. *Neuroimage, 50*(3), 1313-1319.

Hardin, M. G., & Ernst, M. (2009). Functional Brain Imaging of Development-Related Risk and Vulnerability for Substance Use in Adolescents. *Journal of Addiction Medicine, 3*(2), 47-54.

Hasson, R., & Fine, J. G. (2012). Gender Differences Among Children With ADHD on Continuous Performance Tests: A Meta-Analytic Review. *Journal of Attention Disorders, 16*(3), 190-198.

Herjanic, B., & Reich, W. (1997). Development of a structured psychiatric interview for children: Agreement between child and parent on individual symptoms (Reprinted from Journal of Abnormal Child Psychology, vol 10, pg 307-324, 1982). *Journal of Abnormal Child Psychology, 25*(1), 21-31.

Hicks, B. M., Blonigen, D. M., Kramer, M. D., Krueger, R. F., Patrick, C. J., Iacono, W. G., et al. (2007). Gender differences and developmental change in externalizing disorders from late adolescence to early adulthood: A longitudinal twin study. *Journal of Abnormal Psychology, 116*(3), 433-447.

Hommer, D. W., Bjork, J. M., & Gilman, J. M. (2011). Imaging brain response to reward in addictive disorders. *Addiction Reviews, 1216*, 50-61.

Hoogman, M., Aarts, E., Zwiers, M., Slaats-Willemse, D., Naber, M., Onnink, M., et al. (2011). Nitric Oxide Synthase Genotype Modulation of Impulsivity and Ventral Striatal Activity in Adult ADHD Patients and Healthy Comparison Subjects. *American Journal of Psychiatry, 168*(10), 1099-1106.

Ivry, R. B., Spencer, R. M., Zelaznik, H. N., & Diedrichsen, J. (2002). The cerebellum and event timing. *Crebellum: Recent Developments in Cerebellar Research, 978*, 302-317.

Kieling, C., Roman, T., Doyle, A. E., Hutz, M. H., & Rohde, L. A. (2006). Association between DRD4 gene and performance of children with ADHD in a test of sustained attention. *Biological Psychiatry, 60*(10), 1163-1165.

Kirley, A., Hawi, Z., Daly, G., McCarron, M., Mullins, C., Millar, N., et al. (2002). Dopaminergic system genes in ADHD: Toward a biological hypothesis. *Neuropsychopharmacology, 27*(4), 607-619.

Knutson, B., Adams, C. M., Fong, G. W., & Hommer, D. (2001). Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *Journal of Neuroscience, 21*(16), art. no.-RC159.

Knutson, B., Adams, S., Kaiser, E., Walker, J., & Hommer, D. (2000). FMRI visualization of brain activity during anticipation of monetary reward. *Journal of Cognitive Neuroscience*, 56-56.

Knutson, B., & Cooper, J. C. (2005). Functional magnetic resonance imaging of reward prediction. *Current Opinion in Neurology, 18*(4), 411-417.

Knutson, B., Fong, G. W., Adams, C. M., Varner, J. L., & Hommer, D. (2001). Dissociation of reward anticipation and outcome with event-related fMRI. *Neuroreport, 12*(17), 3683-3687.

Knutson, B., Taylor, J., Kaufman, M., Peterson, R., & Glover, G. (2005). Distributed neural representation of expected value. *Journal of Neuroscience, 25*(19), 4806-4812.

Kollins, S. H., Shapiro, S. K., & Abramowitz, A. (1998). Discriminative and participant-rated effects of methylphenidate in children diagnosed with attention deficit hyperactivity disorder (ADHD). *Experimental and Clinical Psychopharmacology, 6*(4), 375-389.

Kopeckova, M., Paclt, I., Petrasek, J., Pacltova, D., Malikova, M., & Zagatova, V. (2008). Some ADHD polymorphisms (in genes DAT1, DRD2, DRD3, DBH, 5-HTT) in case-control study of 100 subjects 6-10 age. *Neuroendocrinology Letters, 29*(2), 246-251.

Krebs, R. M., Schott, B. H., & Duzel, E. (2009). Personality Traits Are Differentially Associated with Patterns of Reward and Novelty Processing in the Human Substantia Nigra/Ventral Tegmental Area. *Biological Psychiatry, 65*(2), 103-110.

Kringelbach, M. L. (2005). The human orbitofrontal cortex: Linking reward to hedonic experience. *Nature Reviews Neuroscience, 6*(9), 691-702.

Kuhn, C., Johnson, M., Thomae, A., Luo, B., Simon, S. A., Zhou, G. Y., et al. (2010). The emergence of gonadal hormone influences on dopaminergic function during puberty. *Hormones and Behavior, 58*(1), 122-137.

Langley, K., Marshall, L., van den Bree, M., Thomas, H., Owen, M., O'Donovan, M., et al. (2004). Association of the dopamine D-4 receptor gene 7-repeat allele with neuropsychological test performance of children with ADHD. *American Journal of Psychiatry, 161*(1), 133-138.

Leckman, J. F., Weissman, M. M., Pauls, D. L., & Kidd, K. K. (1987). Family-Genetic Studies and Identification of Valid Diagnostic Categories in Adult and Child-Psychiatry. *British Journal of Psychiatry, 151*, 39-44.

Lentini, E., Kasahara, M., Arver, S., & Savic, I. (2012). Sex Differences in the Human Brain and the Impact of Sex Chromosomes and Sex Hormones. *Cereb Cortex*.

Levin, H. S., Culhane, K. A., Hartmann, J., Evankovich, K., Mattson, A. J., Harward, H., et al. (1991). Developmental-Changes in Performance on Tests of Purported Frontal-Lobe Functioning. *Developmental Neuropsychology, 7*(3), 377-395.

Levine, E. M., & Kozak, C. (1979). Drug and Alcohol-Use, Delinquency, and Vandalism among Upper Middle-Class Pre-Adolescents and Post-Adolescents. *Journal of Youth and Adolescence, 8*(1), 91-101.

Li, C. S. R., Huang, C., Constable, R. T., & Sinha, R. (2006). Imaging response inhibition in a stop-signal task: Neural correlates independent of signal monitoring and post-response processing. *Journal of Neuroscience, 26*(1), 186-192.

Li, C. s. R., Huang, C. Y., Lin, W. y., & Sun, C. W. V. (2007). Gender differences in punishment and reward sensitivity in a sample of Taiwanese college students. *Personality and Individual Differences, 43*(3), 475-483.

Liston, C., Watts, R., Tottenham, N., Davidson, M. C., Niogi, S., Ulug, A. M., et al. (2006). Frontostriatal microstructure modulates efficient recruitment of cognitive control. *Cerebral Cortex, 16*(4), 553-560.

Liu, X., Hairston, J., Schrier, M., & Fan, J. (2011). Common and distinct networks underlying reward valence and processing stages: A meta-analysis of functional neuroimaging studies. *Neuroscience and Biobehavioral Reviews, 35*(5), 1219-1236.

Logan, G. D., Schachar, R. J., & Tannock, R. (1997). Impulsivity and inhibitory control. *Psychological Science, 8*(1), 60-64.

Lucas, R. E., Diener, E., Grob, A., Suh, E. M., & Shao, L. (2000). Cross-cultural evidence for the fundamental features of extraversion. *Journal of Personality and Social Psychology, 79*(3), 452-468.

Luman, M., van Meel, C. S., Oosterlaan, J., & Geurts, H. M. (2012). Reward and Punishment Sensitivity in Children with ADHD: Validating the Sensitivity to Punishment and Sensitivity to Reward Questionnaire for Children (SPSRQ-C). *Journal of Abnormal Child Psychology, 40*(1), 145-157.

Ma, J., Lei, D., Jin, X., Du, X., Jiang, F., Li, F., et al. (2011). Compensatory brain activation in children with attention deficit/hyperactivity disorder during a simplified Go/No-go task. *Journal of Neural Transmission*, 1-7.

Maldjian, J. A., Laurienti, P. J., & Burdette, J. H. (2004). Precentral gyrus discrepancy in electronic versions of the Talairach atlas. *Neuroimage, 21*(1), 450-455.

Maldjian, J. A., Laurienti, P. J., Kraft, R. A., & Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage, 19*(3), 1233-1239.

Manor, I., Tyano, S., Mel, E., Eisenberg, J., Bachner-Melman, R., Kotler, M., et al. (2002). Family-based and association studies of monoamine oxidase A and attention deficit hyperactivity disorder (ADHD): preferential transmission of the long promoter-region repeat and its association with impaired performance on a continuous performance test (TOVA). *Molecular Psychiatry, 7*(6), 626-632.

May, J. C., Delgado, M. R., Dahl, R. E., Stenger, V. A., Ryan, N. D., Fiez, J. A., et al. (2004). Event-related functional magnetic resonance imaging of reward-related brain circuitry in children and adolescents. *Biological Psychiatry, 55*(4), 359-366.

Meyer-Lindenberg, A., Buckholtz, J. W., Kolachana, B., Hariri, A. R., Pezawas, L., Blasi, G., et al. (2006). Neural mechanisms of genetic risk for impulsivity and violence in humans. *Proceedings of the National Academy of Sciences of the United States of America, 103*(16), 6269-6274.

Munoz, K. E., Hyde, L. W., & Hariri, A. R. (2009). Imaging Genetics. *Journal of the American Academy of Child and Adolescent Psychiatry, 48*(4), 356-361.

Munro, C. A., McCaul, M. E., Wong, D. F., Oswald, L. M., Zhou, Y., Brasic, J., et al. (2006). Sex differences in striatal dopamine release in healthy adults. *Biological Psychiatry, 59*(10), 966-974.

Nees, F., Tzschoppe, J., Patrick, C. J., VollstÃ¤dt-Klein, S., Steiner, S., Poustka, L., et al. (2012). Determinants of Early Alcohol Use In Healthy Adolescents: The Differential Contribution of Neuroimaging and Psychological Factors. *Neuropsychopharmacology*.

Nikolas, M. A., & Burt, S. A. (2010). Genetic and Environmental Influences on ADHD Symptom Dimensions of Inattention and Hyperactivity: A Meta-Analysis. *Journal of Abnormal Psychology, 119*(1), 1-17.

Nikolova, Y. S., Ferrell, R. E., Manuck, S. B., & Hariri, A. R. (2011). Multilocus Genetic Profile for Dopamine Signaling Predicts Ventral Striatum Reactivity. *Neuropsychopharmacology, 36*(9), 1940-1947.

Nixon, S. J., & Parsons, O. A. (1989). Cloninger Tridimensional Theory of Personality - Construct-Validity in a Sample of College-Students. *Personality and Individual Differences, 10*(12), 1261-1267.

Noonan, M. P., Kolling, N., Walton, M. E., & Rushworth, M. F. S. (2012). Re-evaluating the role of the orbitofrontal cortex in reward and reinforcement. *European Journal of Neuroscience, 35*(7), 997-1010.

Ormel, J., Oldehinkel, A. J., Ferdinand, R. F., Hartman, C. A., De Winter, A. F., Veenstra, R., et al. (2005). Internalizing and externalizing problems in adolescence: general and dimension-specific effects of familial loadings and preadolescent temperament traits. *Psychological Medicine, 35*(12), 1825-1835.

Padmala, S., & Pessoa, L. (2010). Interactions between cognition and motivation during response inhibition. *Neuropsychologia, 48*(2), 558-565.

Paloyelis, Y., Mehta, M. A., Faraone, S. V., Asherson, P., & Kuntsi, J. (2012). Striatal Sensitivity During Reward Processing in Attention-Deficit/Hyperactivity Disorder. *Journal of the American Academy of Child and Adolescent Psychiatry, 51*(7), 722-732.

Pelissolo, A., Mallet, L., Baleyte, J. M., Michel, G., Cloninger, C. R., Allilaire, J. F., et al. (2005). The Temperament and Character Inventory-Revised (TCI-R): psychometric characteristics of the French version. *Acta Psychiatrica Scandinavica, 112*(2), 126-133.

Peters, J., Bromberg, U., Schneider, S., Brassen, S., Menz, M., Banaschewski, T., et al. (2011). Lower Ventral Striatal Activation During Reward Anticipation in Adolescent Smokers. *American Journal of Psychiatry, 168*(5), 540-549.

Pinsonneault, J. K., Papp, A. C., & Sadee, W. (2006). Allelic mRNA expression of X-linked monoamine oxidase a (MAOA) in human brain: dissection of epigenetic and genetic factors. *Human Molecular Genetics, 15*(17), 2636-2649.

Pliszka, S. R., Glahn, D. C., Semrud-Clikeman, M., Franklin, C., Perez Iii, R., Xiong, J., et al. (2006). Neuroimaging of inhibitory control areas in children with attention deficit hyperactivity disorder who were treatment naive or in long-term treatment. *American Journal of Psychiatry, 163*(6), 1052-1060.

Plomin, R. (2008). *Behavioral genetics* (5th ed.). New York: Worth.

Pohjalainen, T., Rinne, J. O., Nagren, K., Syvalahti, E., & Hietala, J. (1998). Sex differences in the striatal dopamine D-2 receptor binding characteristics in vivo. *American Journal of Psychiatry, 155*(6), 768-773.

Robbins, T. W., Crockett, M.J. (2010). Role of central serotonin in impulsivity and compulsivity: Comparative studies in animals and humans. In C. P. Muller, Jacobs, B. (Ed.), *Handbook of the behavioral neurobiology of serotonin* (First Edition ed., pp. 415-427). London: Elsevier BV.

Romer, D., & Hennessy, M. (2007). A biosocial-affect model of adolescent sensation seeking: The role of affect evaluation and peer-group influence in adolescent drug use. *Prevention Science, 8*(2), 89-101.

Rommelse, N. N. J., Altink, M. E., Arias-Vasquez, A., Buschgens, C. J. M., Fliers, E., Faraone, S. V., et al. (2008). Differential Association Between MAOA, ADHD and Neuropsychological Functioning in Boys and Girls. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics, 147B*(8), 1524-1530.

Rubia, K. (2011). "Cool" Inferior Frontostriatal Dysfunction in Attention-Deficit/Hyperactivity Disorder Versus "Hot" Ventromedial Orbitofrontal-Limbic Dysfunction in Conduct Disorder: A Review. *Biological Psychiatry, 69*(12), E69-E87.

Rubia, K., Halari, R., Smith, A. B., Mohammed, M., Scott, S., Giampietro, V., et al. (2008). Dissociated functional brain abnormalities of inhibition in boys with pure conduct disorder and in boys with pure attention deficit hyperactivity disorder. *American Journal of Psychiatry, 165*(7), 889-897.

Rubia, K., Smith, A. B., Brammer, M. J., & Taylor, E. (2003). Right inferior prefrontal cortex mediates response inhibition while mesial prefrontal cortex is responsible for error detection. *Neuroimage, 20*(1), 351-358.

Rubia, K., Smith, A. B., Brammer, M. J., Toone, B., & Taylor, E. (2005). Abnormal brain activation during inhibition and error detection in medication-naive adolescents with ADHD. *American Journal of Psychiatry, 162*(6), 1067-1075.

Rubia, K., Smith, A. B., Taylor, E., & Brammer, M. (2007). Linear age-correlated functional development of right inferior fronto-striato-cerebellar networks during response inhibition and anterior Cingulate during error-related processes. *Human Brain Mapping, 28*(11), 1163-1177.

Rushworth, M., Behrens, T., & Walton, M. (2008). The anterior cingulate cortex in learning and reward-guided decision making. *International Journal of Psychology, 43*(3-4), 355-355.

Rushworth, M. F. S., & Behrens, T. E. J. (2008). Choice, uncertainty and value in prefrontal and cingulate cortex. *Nature Neuroscience, 11*(4), 389-397.

Samaha, A. N., & Robinson, T. E. (2005). Why does the rapid delivery of drugs to the brain promote addiction? *Trends in Pharmacological Sciences, 26*(2), 82-87.

Sasik, R., Calvo, E., & Corbeil, J. (2002). Statistical analysis of high-density oligonucleotide arrays: a multiplicative noise model. *Bioinformatics, 18*(12), 1633-1640.

Savic, I. (2010). *Sex Differences in the Human Brain, Their Underpinnings and Implications* (First ed.). New York, USA: Elsevier.

Scheres, A., Milham, M. P., Knutson, B., & Castellanos, F. X. (2007a). Ventral striatal hyporesponsiveness during reward anticipation in attention-deficit/hyperactivity disorder. *Biological Psychiatry, 61*(5), 720-724.

Scheres, A., Milham, M. P., Knutson, B., & Castellanos, F. X. (2007b). Ventral striatal hyporesponsiveness during reward anticipation in attention-deficit/hyperactivity disorder. *Biol Psychiatry, 61*(5), 720-724.

Schneider, S., Peters, J., Bromberg, U., Brassen, S., Miedl, S. F., Banaschewski, T., et al. (2012). Risk Taking and the Adolescent Reward System: A Potential Common Link to Substance Abuse. *American Journal of Psychiatry, 169*(1), 39-46.

Schott, B. H., Minuzzi, L., Krebs, R. M., Elmenhorst, D., Lang, M., Winz, O. H., et al. (2008). Mesolimbic Functional Magnetic Resonance Imaging Activations during Reward Anticipation Correlate with Reward-Related Ventral Striatal Dopamine Release. *Journal of Neuroscience, 28*(52), 14311-14319.

Schultz, W., Dayan, P., & Montague, P. R. (1997). A neural substrate of prediction and reward. *Science, 275*(5306), 1593-1599.

Schulz, K. P., Fan, J., Tang, C. Y., Newcorn, J. H., Buchsbaum, M. S., Cheung, A. M., et al. (2004). Response inhibition in adolescents diagnosed with attention deficit hyperactivity disorder during childhood: An event-related fMRI study. *American Journal of Psychiatry, 161*(9), 1650-1657.

Schulz, K. P., Newcorn, J. H., Fan, J., Tang, C. Y., & Halperin, J. M. (2005). Brain activation gradients in ventrolateral prefrontal cortex related to persistence of ADHD in adolescent boys. *Journal of the American Academy of Child and Adolescent Psychiatry, 44*(1), 47-54.

Schumann, G., Loth, E., Banaschewski, T., Barbot, A., Barker, G., Buchel, C., et al. (2010). The IMAGEN study: reinforcement-related behaviour in normal brain function and psychopathology. *Molecular Psychiatry, 15*(12), 1128-1139.

Sescousse, G., Redoute, J., & Dreher, J. C. (2010). The Architecture of Reward Value Coding in the Human Orbitofrontal Cortex. *Journal of Neuroscience, 30*(39), 13095-13104.

Shih, J. C. (2004). Cloning, after cloning, knock-out mice, and physiological functions of MAO A and B. *Neurotoxicology, 25*(1-2), 21-30.

Shiraishi, H., Suzuki, A., Fukasawa, T., Aoshima, T., Ujiie, Y., Ishii, G., et al. (2006). Monoamine oxidase A gene promoter polymorphism affects novelty seeking and reward dependence in healthy study participants. *Psychiatric Genetics, 16*(2), 55-58.

Snopek, M., Hublova, V., Porubanova, M., & Blatny, M. (2012). Psychometric properties of the Temperament and Character Inventory-Revised (TCI-R) in Czech adolescent sample. *Comprehensive Psychiatry, 53*(1), 71-80.

Solanto, M. V., Abikoff, H., Sonuga-Barke, E., Schachar, R., Logan, G. D., Wigal, T., et al. (2001). The ecological validity of delay aversion and response inhibition as measures of impulsivity in AD/HD: A supplement to the NIMH

multimodal treatment study of AD/HD. *Journal of Abnormal Child Psychology, 29*(3), 215-228.

Sonuga-Barke, E., Bitsakou, P., & Thompson, M. (2010). Beyond the Dual Pathway Model: Evidence for the Dissociation of Timing, Inhibitory, and Delay-Related Impairments in Attention-Deficit/Hyperactivity Disorder. *Journal of the American Academy of Child and Adolescent Psychiatry, 49*(4), 345-355.

Sonuga-Barke, E. J. S. (2002). Psychological heterogeneity in AD/HD - a dual pathway model of behaviour and cognition. *Behavioural Brain Research, 130*(1-2), 29-36.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews, 24*(4), 417-463.

Spreckelmeyer, K. N., Krach, S., Kohls, G., Rademacher, L., Irmak, A., Konrad, K., et al. (2009). Anticipation of monetary and social reward differently activates mesolimbic brain structures in men and women. *Social Cognitive and Affective Neuroscience, 4*(2), 158-165.

Steinberg, L., Albert, D., Cauffman, E., Banich, M., Graham, S., & Woolard, J. (2008). Age Differences in Sensation Seeking and Impulsivity as Indexed by Behavior and Self-Report: Evidence for a Dual Systems Model. *Developmental Psychology, 44*(6), 1764-1778.

Stevens, M. C., Kiehl, K. A., Pearlson, G. D., & Calhoun, V. D. (2007). Functional neural networks underlying response inhibition in adolescents and adults. *Behav Brain Res, 181*(1), 12-22.

Stoy, M., Schlagenhauf, F., Schlochtermeier, L., Wrase, J., Knutson, B., Lehmkuhl, U., et al. (2011). Reward processing in male adults with childhood ADHD-a comparison between drug-naive and methylphenidate-treated subjects. *Psychopharmacology, 215*(3), 467-481.

Strohle, A., Stoy, M., Wrase, J., Schwarzer, S., Schlagenhauf, F., Huss, M., et al. (2008). Reward anticipation and outcomes in adult males with attention-deficit/hyperactivity disorder. *Neuroimage, 39*(3), 966-972.

Swick, D., Ashley, V., & Turken, U. (2011). Are the neural correlates of stopping and not going identical? Quantitative meta-analysis of two response inhibition tasks. *Neuroimage, 56*(3), 1655-1665.

Tamm, L., Menon, V., & Reiss, A. L. (2002). Maturation of brain function associated with response inhibition. *Journal of the American Academy of Child and Adolescent Psychiatry, 41*(10), 1231-1238.

Tarazi, F. I., Tomasini, E. C., & Baldessarini, R. J. (1999). Dopamine D-1-D-2-like and D-4 receptors follow a similar course of postnatal development in cortical and striatolimbic brain regions. *Journal of Neurochemistry, 73*, S172-S172.

Teicher, M. H., Andersen, S. L., & Hostetter, J. C. (1995). Evidence for Dopamine-Receptor Pruning between Adolescence and Adulthood in Striatum but Not Nucleus-Accumbens. *Developmental Brain Research, 89*(2), 167-172.

Telzer, E. H., Fuligni, A. J., Lieberman, M. D., & Galvan, A. Ventral Striatum Activation to Prosocial Rewards Predicts Longitudinal Declines in Adolescent Risk Taking. *Developmental Cognitive Neuroscience*(0).

Thoma, P., Bellebaum, C., Koch, B., Schwarz, M., & Daum, I. (2008). The Cerebellum Is Involved in Reward-based Reversal Learning. *Cerebellum, 7*(3), 433-443.

Torrubia, R., Avila, C., Molto, J., & Caseras, X. (2001). The Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ) as a measure

of Gray's anxiety and impulsivity dimensions. *Personality and Individual Differences, 31*(6), 837-862.

Tripp, G., & Alsop, B. (2001). Sensitivity to reward delay in children with attention deficit hyperactivity disorder (ADHD). *Journal of Child Psychology and Psychiatry and Allied Disciplines, 42*(5), 691-698.

Tripp, G., & Wickens, J. R. (2008). Research review: dopamine transfer deficit: a neurobiological theory of altered reinforcement mechanisms in ADHD. [Research Support, Non-U.S. Gov't

Review]. *J Child Psychol Psychiatry, 49*(7), 691-704.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., et al. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage, 15*(1), 273-289.

van Honk, J., Schutter, D. J. L. G., Hermans, E. J., Putman, P., Tuiten, A., & Koppeschaar, H. (2004). Testosterone shifts the balance between sensitivity for punishment and reward in healthy young women. *Psychoneuroendocrinology, 29*(7), 937-943.

Vanni-Mercier, G., Mauguiere, F., Isnard, J., & Dreher, J. C. (2009). The Hippocampus Codes the Uncertainty of Cue-Outcome Associations: An Intracranial Electrophysiological Study in Humans. *Journal of Neuroscience, 29*(16), 5287-5294.

Viding, E., Williamson, D. E., & Hariri, A. R. (2006). Developmental imaging genetics: Challenges and promises for translational research. *Development and Psychopathology, 18*(3), 877-892.

Williams, B. R., Ponesse, J. S., Schachar, R. J., Logan, G. D., & Tannock, R. (1999). Development of inhibitory control across the life span. *Developmental Psychology, 35*(1), 205-213.

Wills, T. A., Vaccaro, D., & McNamara, G. (1994). Novelty seeking, risk taking, and related constructs as predictors of adolescent substance use: An application of Cloninger's theory. *Journal of Substance Abuse, 6*(1), 1-20.

Wittmann, B. C., Daw, N. D., Seymour, B., & Dolan, R. J. (2008). Striatal activity underlies novelty-based choice in humans. *Neuron, 58*(6), 967-973.

Woerner, W., Becker, A., Friedrich, C., Klasen, H., Goodman, R., & Rothenberger, A. (2002). [Normal values and evaluation of the German parents' version of Strengths and DIfficulties Questionnaire (SDQ): Results of a representative field study]. *Z Kinder Jugendpsychiatr Psychother, 30*(2), 105-112.

Yacubian, J., Glascher, J., Schroeder, K., Sommer, T., Braus, D. F., & Buchel, C. (2006). Dissociable systems for gain- and loss-related value predictions and errors of prediction in the human brain (vol 26, pg 9530, 2006). *Journal of Neuroscience, 26*(39).

Zimic, J. I., & Jukic, V. (2012). Familial Risk Factors Favoring Drug Addiction Onset. *Journal of Psychoactive Drugs, 44*(2), 173-185.

Zuckerman, M. (1979). *Sensation seeking : beyond the optimal level of arousal*. Hillsdale, N.J ; New York

London: Erlbaum ;

Distributed by Wiley.

Zuckerman, M., & Kuhlman, D. M. (2000). Personality and risk-taking: Common biosocial factors. *Journal of Personality, 68*(6), 999-1029.

9 SUPPLEMENTARY TABLES AND APPENDICES

9.1 SUPPLEMENTARY TABLE 1

Brain areas activated by anticipation and feedback (FDR $p < 0.05$ and a minimum cluster size of 10 voxels) in meta-analysis by Liu et al. (2011)

Region	***MNI-Coordinates***	***Cluster size (k)***
Anticipation		
Nucleus Accumbens	12 10 -4	7960
Nucleus Accumbens	-12 10 -6	
Insula	38 20 -8	
Insula	-32 18 -6	
Thalamus	4 -12 12	
Thalamus	-10 -22 12	
Brain Stem	8 -18 -10	
Brain Stem	-4 -24 -6	
Putamen	24 4 0	
Supplementary Motor Area	2 8 48	2258
Supplementary Motor Area	-2 -6 50	
Anterior Cingulate Cortex	2 24 40	
Anterior Cingulate Cortex	4 38 38	
Anterior Cingulate Cortex	2 24 34	
Medial Orbitofrontal Cortex	-2 50 -16	450
Inferior Parietal Lobule	-28 -58 50	327
Middle Frontal Gyrus	40 28 34	192
Superior Parietal Lobule	34 -52 52	131
Middle Frontal Gyrus	-26 4 52	119
Precentral Gyrus	-44 6 30	95
Posterior Cingulate Cortex	0 -30 32	94
Feedback		
Nucleus Accumbens	12 10 -6	11322
Nucleus Accumbens	-10 8 -4	
Medial Orbitofrontal Cortex	-2 56 -6	
Medial Orbitofrontal Cortex	2 48 -14	
Amygdala	26 0 -16	
Insula	36 22 -8	
Insula	-28 24 -8	
Thalamus	4 -16 6	
Anterior Cingulate Cortex	8 24 32	
Supplementary Motor Area	4 22 52	
Frontal Pole	-18 40 -16	
Posterior Cingulate Cortex	0 -22 32	345
Superior Frontal Gyrus	-24 30 48	150

Supplementary Motor Area	2 -6 50	147
Inferior Frontal Gyrus	-54 18 16	113
Occipital Pole	-32 -94 -12	111
Middle Frontal Gyrus	44 36 28	110

9.2 SUPPLEMENTARY TABLE 2

Demographics of boys who had completed the SST task (n = 143): Means, standard deviations and ranges are presented below. We found no significant genotype differences in age ($t = 0.16$, $p = 0.69$), verbal (VIQ: $t = 0.73$, $p = 0.40$) or performance IQ (PIQ: $t = 0.03$, $p = 0.87$) after controlling for study site.

	A hemizygotes N = 52	**G hemizygotes** N = 91	**Total** N = 143
Age (yrs)	14.5 ± 0.4 *(13.6-15.5)*	14.5 ± 0.4 *(13.6-15.6)*	14.5 ± 0.4 *(13.6-15.6)*
VIQ	118.3 ± 14.4 *(83-150)*	115.0 ± 14.6 *(87-155)*	116.2 ± 14.6 *(83-155)*
PIQ	108.2 ± 14.9 *(81-149)*	108.5 ± 12.2 *(81-135)*	108.4 ± 13.2 *(81-149)*
ADHD-Symptoms	2.5 ± 1.8 *(0-7)*	3.2 ± 2.4 *(0-10)*	2.9 ± 2.2 *(0-10)*

ADHD = Attention Deficit Hyperactivity Disorder, VIQ = Verbal IQ, PIQ = Reasoning IQ

9.3 APPENDIX 1: STRENGTHS AND DIFFICULTIES QUESTIONNAIRE

Please refer to www.dawba.com for further details and the complete version of the questionnaires including wording of items and responses, as well as scoring.

1) Variable labels

```
variable labels dawbaID 'ID'.
variable labels age 'Age'.
variable labels gender 'Gender'.
variable labels p1startdate 'Data last entered (Parent1)'.
variable labels p1type 'Informant (Parent1)'.
variable labels p1consid 'SDQ: Considerate (Parent1)'.
variable labels p1restles 'SDQ: Restless (Parent1)'.
variable labels p1somatic 'SDQ: Headache, stomach-ache (Parent1)'.
variable labels p1shares 'SDQ: Shares (Parent1)'.
variable labels p1tantrum 'SDQ: Irritable (Parent1)'.
variable labels p1loner 'SDQ: Solitary (Parent1)'.
variable labels p1obeys 'SDQ: Obedient (Parent1)'.
variable labels p1worries 'SDQ: Worries (Parent1)'.
variable labels p1caring 'SDQ: Helpful (Parent1)'.
variable labels p1fidgety 'SDQ: Fidgety (Parent1)'.
variable labels p1friend 'SDQ: Has good friend (Parent1)'.
variable labels p1fights 'SDQ: Fights, bullies (Parent1)'.
variable labels p1unhappy 'SDQ: Unhappy (Parent1)'.
variable labels p1popular 'SDQ: Popular (Parent1)'.
variable labels p1distrac 'SDQ: Poor concentration (Parent1)'.
variable labels p1clingy 'SDQ: Anxious in new situations (Parent1)'.
variable labels p1kind 'SDQ: Kind to younger children (Parent1)'.
variable labels p1lies 'SDQ: Lies, cheats (Parent1)'.
variable labels p1bullied 'SDQ: Victimised (Parent1)'.
variable labels p1helpout 'SDQ: Volunteers to help (Parent1)'.
variable labels p1reflect 'SDQ: Reflective (Parent1)'.
variable labels p1steals 'SDQ: Steals (Parent1)'.
variable labels p1oldbest 'SDQ: Relates better to adults than peers (Parent1)'.
variable labels p1afraid 'SDQ: Fears (Parent1)'.
variable labels p1attends 'SDQ: Good attention (Parent1)'.
variable labels p1ebddiff 'SDQ: Is there a problem? (Parent1)'.
variable labels p1chronic 'SDQ: Duration (months) (Parent1)'.
variable labels p1distres 'SDQ: Distress (Parent1)'.
variable labels p1imphome 'SDQ: Impact on family life (Parent1)'.
variable labels p1impfrie 'SDQ: Impact on friendship (Parent1)'.
variable labels p1impclas 'SDQ: Impact on learning (Parent1)'.
variable labels p1impleis 'SDQ: Impact on leisure (Parent1)'.
variable labels p1burden 'SDQ: Burden (Parent1)'.
variable labels p1ebdtot 'SDQ: Total difficulties score (Parent1)'.
variable labels p1emotion 'SDQ: Emotional symptoms score (Parent1)'.
variable labels p1conduct 'SDQ: Conduct problems score (Parent1)'.
```

variable labels p1hyper 'SDQ: Hyperactivity score (Parent1)'.
variable labels p1peer 'SDQ: Peer problems score (Parent1)'.
variable labels p1prosoc 'SDQ: Prosocial score (Parent1)'.
variable labels p1impact 'SDQ: Impact score (Parent1)'.

2) value labels

value labels gender 1 'Male' 2 'Female'.
value labels p1type 1 'Parent' 2 'Mother' 3 'Father' 4 'Both parents' 5 'Stepmother' 6 'Stepfather' 7 'Foster mother' 8 'Foster father' 9 'Grandparent' 10 'Other relative' 11 'Residential care worker'.
value labels p1consid 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1restles 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1somatic 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1shares 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1tantrum 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1loner 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1obeys 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1worries 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1caring 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1fidgety 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1friend 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1fights 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1unhappy 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1popular 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1distrac 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1clingy 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1kind 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1lies 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1bullied 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1helpout 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1reflect 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1steals 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1oldbest 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1afraid 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1attends 0 'Not True' 1 'Partly True' 2 'Certainly True'.
value labels p1ebddiff 0 'No' 1 'Yes - minor difficulties' 2 'Yes - definite difficulties' 3 'Yes - severe difficulties'.
value labels p1chronic 0 'Less than 1 month' 1 '1-5 months' 2 '6-12 months' 3 'Over a year'.
value labels p1distres 0 'Not at all' 1 'A little' 2 'A medium amount' 3 'A great deal'.
value labels p1imphome 0 'Not at all' 1 'A little' 2 'A medium amount' 3 'A great deal'.
value labels p1impfrie 0 'Not at all' 1 'A little' 2 'A medium amount' 3 'A great deal'.
value labels p1impclas 0 'Not at all' 1 'A little' 2 'A medium amount' 3 'A great deal'.
value labels p1impleis 0 'Not at all' 1 'A little' 2 'A medium amount' 3 'A great deal'.
value labels p1burden 0 'Not at all' 1 'A little' 2 'A medium amount' 3 'A great deal'.

Summary variables (youth and parent) - Variable labels and description

1) Variable labels

variable labels sdqed 'SDQ: Emotional disorder (Computer prediction)'.
variable labels sdqcd 'SDQ: Behavioural disorder (Computer prediction)'.
variable labels sdqhk 'SDQ: Hyperactivity disorder (Computer prediction)'.
variable labels sdqcase 'SDQ: Any disorder (Computer prediction)'.

2) Value labels

value labels sdqed 0 'unlikely' 1 'possible' 2 'probable'.
value labels sdqcd 0 'unlikely' 1 'possible' 2 'probable'.
value labels sdqhk 0 'unlikely' 1 'possible' 2 'probable'.
value labels sdqcase 0 'unlikely' 1 'possible' 2 'probable'.

9.4 APPENDIX 2: TEMPERAMENT AND CHARACTER INVENTORY

1) Variable labels

VARIABLE LABELS C.tci001 'I often try new things just for fun or thrills, even if most people think it is a waste of time.'.
VARIABLE LABELS C.tci010 'I often do things based on how I feel at the moment without thinking about how they were done in the past.'.
VARIABLE LABELS C.tci014 'I am much more reserved and controlled than most people.'.
VARIABLE LABELS C.tci024 'I often spend money until I run out of cash or get into debt from using too much credit.'.
VARIABLE LABELS C.tci044 'I like it when people can do whatever they want without strict rules and regulations.'.
VARIABLE LABELS C.tci047 'I usually think about all the facts in detail before I make a decision.'.
VARIABLE LABELS C.tci051 'I am usually able to get other people to believe me, even when I know that what I am saying is exaggerated or untrue.'.
VARIABLE LABELS C.tci059 'I prefer spending money rather than saving it.'.
VARIABLE LABELS C.tci063 'I usually demand very good practical reasons before I am willing to change my old ways of doing things.'.
VARIABLE LABELS C.tci071 'I often follow my instincts, hunches, or intuition without thinking through all the details.'.
VARIABLE LABELS C.tci077 'Even when most people feel it is not important, I often insist on things being done in a strict and orderly way.'.
VARIABLE LABELS C.tci053 'I have a reputation as someone who is very practical and does not act on emotion.'.
VARIABLE LABELS C.tci102 'I like to make quick decisions so I can get on with what has to be done.'.
VARIABLE LABELS C.tci104 'I like to explore new ways to do things.'.
VARIABLE LABELS C.tci105 'I enjoy saving money more than spending it on entertainment or thrills.'.
VARIABLE LABELS C.tci109 'I often break rules and regulations when I think I can get away with it.'.
VARIABLE LABELS C.tci122 'When nothing new is happening, I usually start looking for something that is thrilling or exciting.'.
VARIABLE LABELS C.tci123 'I like to think about things for a long time before I make a decision.'.
VARIABLE LABELS C.tci135 'I can usually do a good job of stretching the truth to tell a funnier story or to play a joke on someone.'.
VARIABLE LABELS C.tci139 'I am better at saving money than most people.'.
VARIABLE LABELS C.tci145 'I am slower than most people to get excited about new ideas and activities.'.
VARIABLE LABELS C.tci155 'Some people think I am too stingy or tight with my money.'.
VARIABLE LABELS C.tci156 'I like old "tried and true" ways of doing things much better than trying "new and improved" ways.'.
VARIABLE LABELS C.tci159 'I am not very good at talking my way out of trouble when I am caught doing something wrong.'.

VARIABLE LABELS C.tci165 'In conversations I am much better as a listener than as a talker.'.
VARIABLE LABELS C.tci170 'I have some trouble telling a lie, even when it is meant to spare someone elses feelings.'.
VARIABLE LABELS C.tci172 'It is hard for me to enjoy spending money on myself, even when I have saved plenty of money.'.
VARIABLE LABELS C.tci176 'I like to stay at home better than to travel or explore new places.'.
VARIABLE LABELS C.tci179 'I like to read everything when I am asked to sign any papers.'.
VARIABLE LABELS C.tci193 'I hate to make decisions based only on my first impressions.'.
VARIABLE LABELS C.tci205 'I hate to change the way I do things, even if many people tell me there is a new and better way to do it.'.
VARIABLE LABELS C.tci210 'I like to pay close attention to details in everything I do.'.
VARIABLE LABELS C.tci215 'Because I so often spend too much money on impulse, it is hard for me to save money - even for special plans like a vacation.'.
VARIABLE LABELS C.tci222 'It is fun for me to buy things for myself.'.

2) Value labels

VALUE LABELS C.tci001 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci010 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci014 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci024 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci044 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci047 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci051 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci059 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci063 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci071 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci077 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci053 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci102 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .

VALUE LABELS C.tci104 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci105 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci109 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci122 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci123 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci135 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci139 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci145 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci155 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci156 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci159 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci165 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci170 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci172 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci176 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci179 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci193 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci205 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci210 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .
VALUE LABELS C.tci215 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci222 1 'definitely false' 2 'mostly false' 3 'neither true or false' 4 'mostly true' 5 'definitely true' .
VALUE LABELS C.tci239 5 'definitely false' 4 'mostly false' 3 'neither true or false' 2 'mostly true' 1 'definitely true' .

Summary variables - Variable labels and description

- exploratory excitability vs. stoic rigidity

VARIABLE LABES C.tci_excit 'CHILD NS1: exploratory excitability vs. stoic rigidity total =sum(C.tci001, C.tci063, C.tci053, C.tci104, C.tci122, C.tci145, C.tci156, C.tci165, C.tci176, C.tci205)'.

- impulsiveness vs. reflection

VARIABLE LABES C.tci_imp 'CHILD NS2: impulsiveness vs. reflection total =sum(C.tci010, C.tci047, C.tci071, C.tci102, C.tci123, C.tci179, C.tci193, C.tci210, C.tci239)'.

- extravagance vs. reserve

VARIABLE LABES C.tci_extra 'CHILD NS3: extravagance vs. reserve total =sum(C.tci014, C.tci024, C.tci059, C.tci105, C.tci139, C.tci155, C.tci172, C.tci215, C.tci222)'.

- disorderliness vs. regimentation

VARIABLE LABELS C.tci_diso 'CHILD NS4: disorderliness vs. regimentation total =sum (C.tci044, C.tci051, C.tci077, C.tci109, C.tci135, C.tci159, C.tci170)'.

- Total Novelty Seeking score

VARIABLE LABELS C.tci_novseek 'CHILD NS: NOVERLTYSEEKING TOTAL=sum(C.tci_excit to C.tci_diso)' .

9.5 APPENDIX 3: THE PUBERTY DEVELOPMENT SCALE

PDS

Items

1) Variable labels

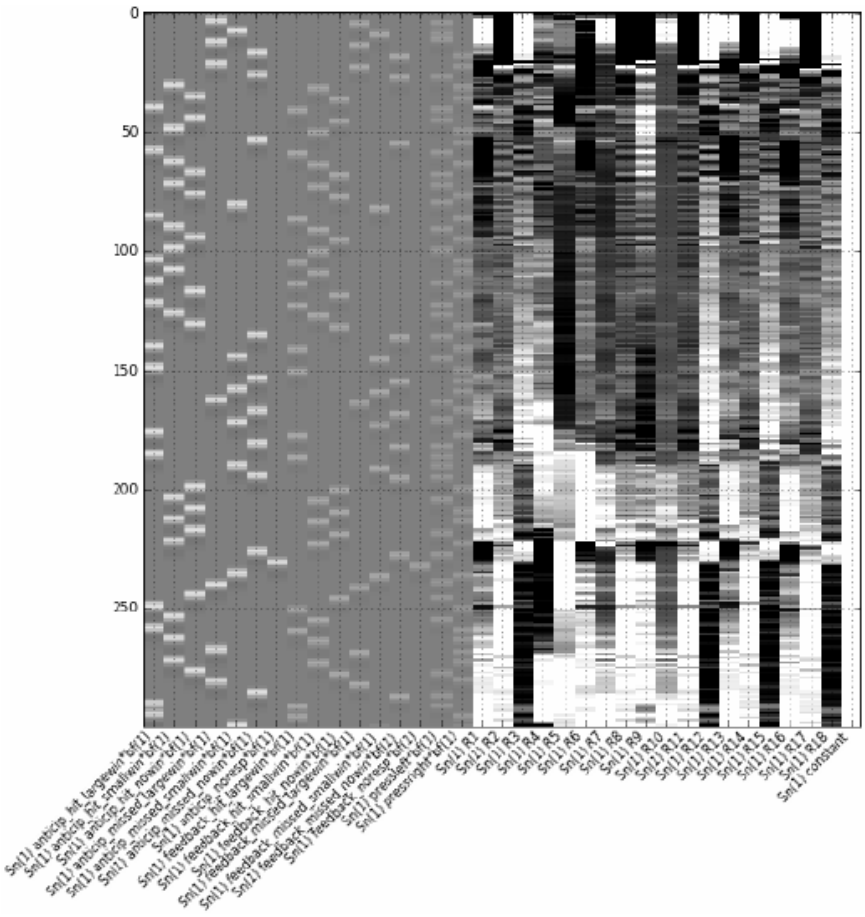
VARIABLE LABELS a8_f 'Would you say that your growth in height: …?'.
VARIABLE LABELS a9_f 'And how about the growth of body hair (body hair means underarm and pubic hair), would you say that your body hair has:…?'.
VARIABLE LABELS a10_f 'Have you noticed any skin changes, especially pimples?'.
VARIABLE LABELS a11_f 'Have your breasts begun to grow?'.
VARIABLE LABELS a12a_f 'Have you begun to menstruate?'.
VARIABLE LABELS a12b_f 'How old were you when you had your first period?'.
VARIABLE LABELS a13_f 'Do you think your development is any earlier or later than most other girls your age?'.
VARIABLE LABELS a8_m 'Would you say that your growth in height: …?'.
VARIABLE LABELS a9_m 'And how about the growth of body hair (body hair means underarm and pubic hair), would you say that your body hair has:…?'.
VARIABLE LABELS a10_m 'Have you noticed any skin changes, especially pimples?'.
VARIABLE LABELS a11_m 'Have you noticed a deepening of your voice?'.
VARIABLE LABELS a12_m 'Have you begun to grow hair on your face?'.
VARIABLE LABELS a13_m 'Do you think your development is any earlier or later than most other boys your age?'.

2) Value labels

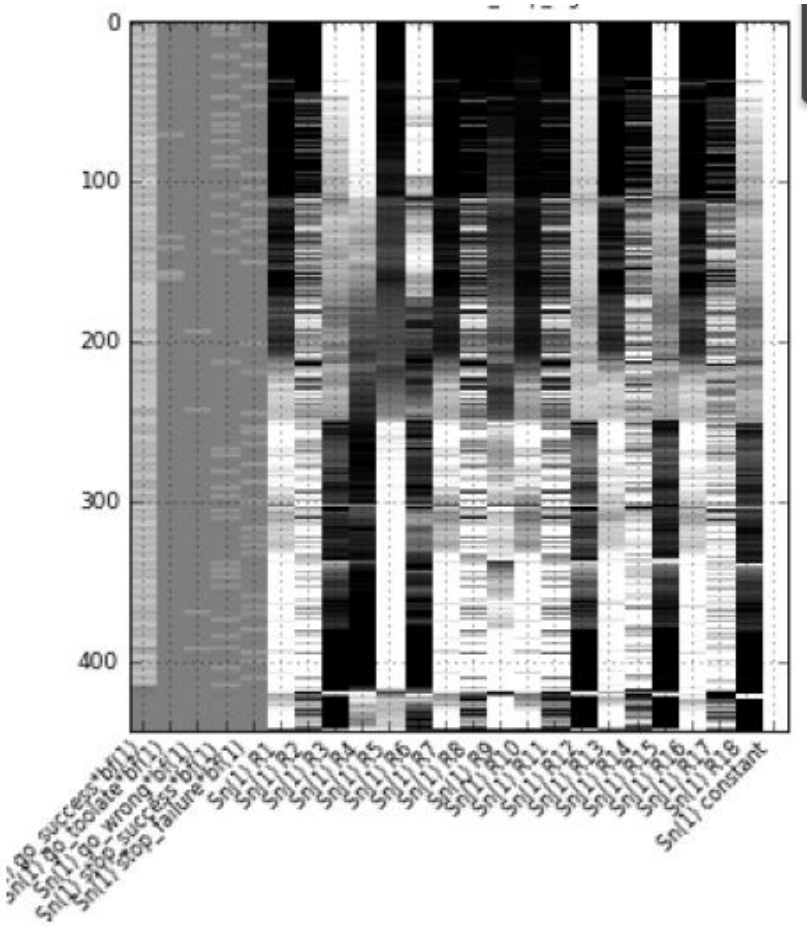
VALUE LABELS a8_f 1 'Has not yet begun to spurt (spurt means more growth than usual)' 2 'Has barely started' 3 'Is definitely underway' 4 'Seems completed' .
VALUE LABELS a9_f 1 'Not yet started growing' 2 'Has barely started growing' 3 'Is definitely underway' 4 'Seems completed' .
VALUE LABELS a10_f 1 'Not yet started showing changes' 2 'Have barely started showing changes' 3 'Skin changes are definitely underway' 4 'Skin changes seem completed' .
VALUE LABELS a11_f 1 'Not yet started growing' 2 'Has barely started changing' 3 'Breast growth is definitely underway' 4 'Breast growth seems completed' .
VALUE LABELS a12a_f 1 'Yes' 0 'No' .
VALUE LABELS a12b_f 10 '10 years or younger' 11 '11' 12 '12' 13 '13' 14 '14' .
VALUE LABELS a13_f 5 'Much earlier' 4 'Somewhat earlier' 3 'About the same' 2 'Somewhat later' 1 'Much later' .
VALUE LABELS a8_m 1 'Has not yet begun to spurt (spurt means more growth than usual)' 2 'Has barely started' 3 'Is definitely underway' 4 'Seems completed' .
VALUE LABELS a9_m 1 'Not yet started growing' 2 'Has barely started growing' 3 'Is definitely underway' 4 'Seems completed' .
VALUE LABELS a10_m 1 'Not yet started showing changes' 2 'Have barely started showing changes' 3 'Skin changes are definitely underway' 4 'Skin changes seem completed' .
VALUE LABELS a11_m 1 'Not yet started changing' 2 'Has barely started changing' 3 'Voice change is definitely underway' 4 'Voice change seems completed' .
VALUE LABELS a12_m 1 'Not yet started growing hair' 2 'Has barely started growing hair' 3 'Facial hair growth is definitely underway' 4 'Facial hair growth seems completed' .
VALUE LABELS a13_m 5 'Much earlier' 4 'Somewhat earlier' 3 'About the same' 2 'Somewhat later' 1 'Much later' .

9.6 APPENDIX 4: FIRST LEVEL MODELS OF THE MID AND SST

First level model of the MID task, as created by Neurospin. The model includes 16 conditions and 18 movement regressors as displayed below. The conditions referred to the two levels of reward anticipation (i.e. reward anticipation and reward feedback), the three levels of reward received (no, low, high), whether the individual was presented with a cue on the left or right side of the screen and whether the individual hit, missed or did not respond to the target. Estimated movement was added to the design matrix in the form of 18 additional columns (3 translations, 3 rotations, 3 quadratic and 3 cubic translations, 3 translations shifted 1 TR before, and 3 translations shifted 1 TR later). The regressors modeling the experimental conditions were convolved using SPM's default Hemodynamic Response Function.



First level model of the stop signal task as created by Neurospin. The model contains 5 conditions and 18 regressors as shown below. The conditions of this model included go success, go too late, go wrong, stop success, stop failure. The movement regressors were modelled as described above.

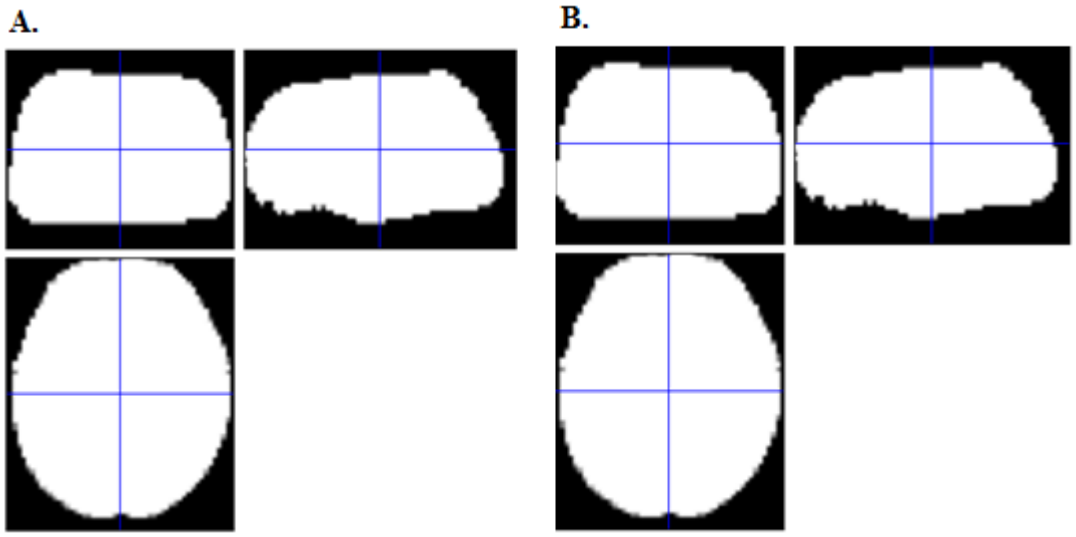


9.7 APPENDIX 5: MASKS FROM SECOND-LEVEL ANALYSES

Chapter 3:

A. Mask.img for anticipation large win vs. no win (n = 1243, voxels in mask: 67210),

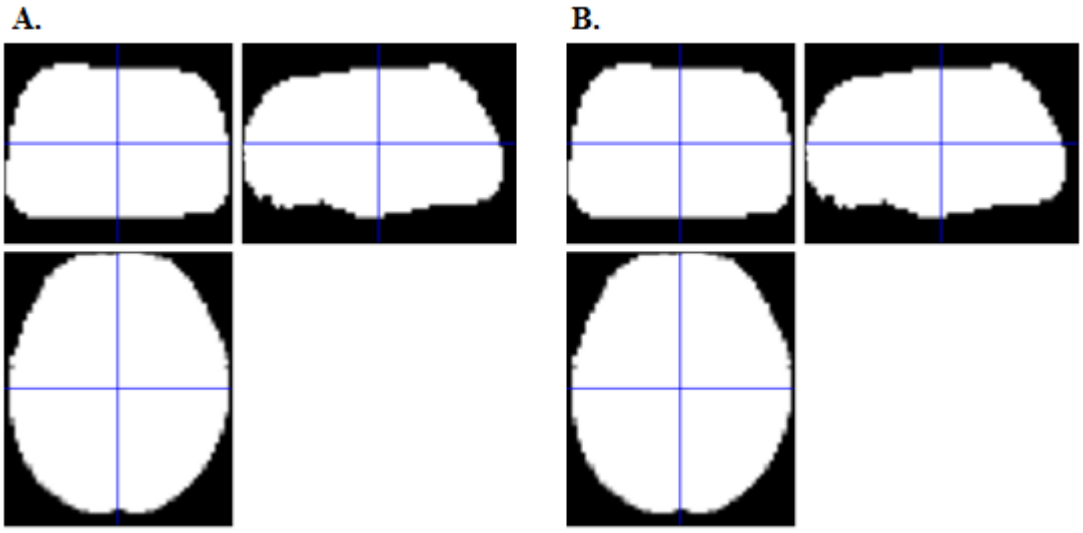
B. Mask.img for feedback large win vs. no win (n = 1243, voxels in mask: 67210)



Chapter 4:

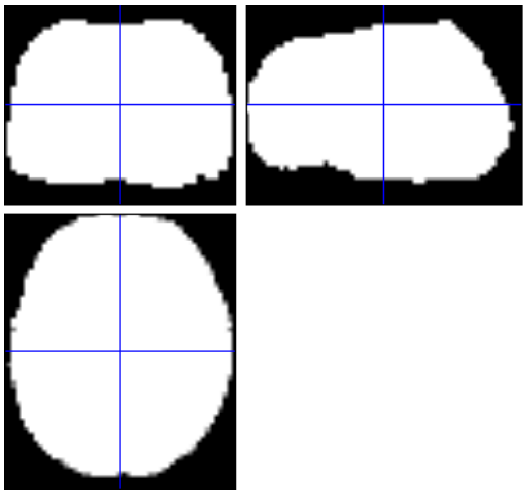
A.Mask.img for anticipation large win vs. no win (n = 1234, voxels in mask: 67341),

B. Mask.img for feedback large win vs. no win (n = 1234, voxels in mask: 67341)



Chapter 5:

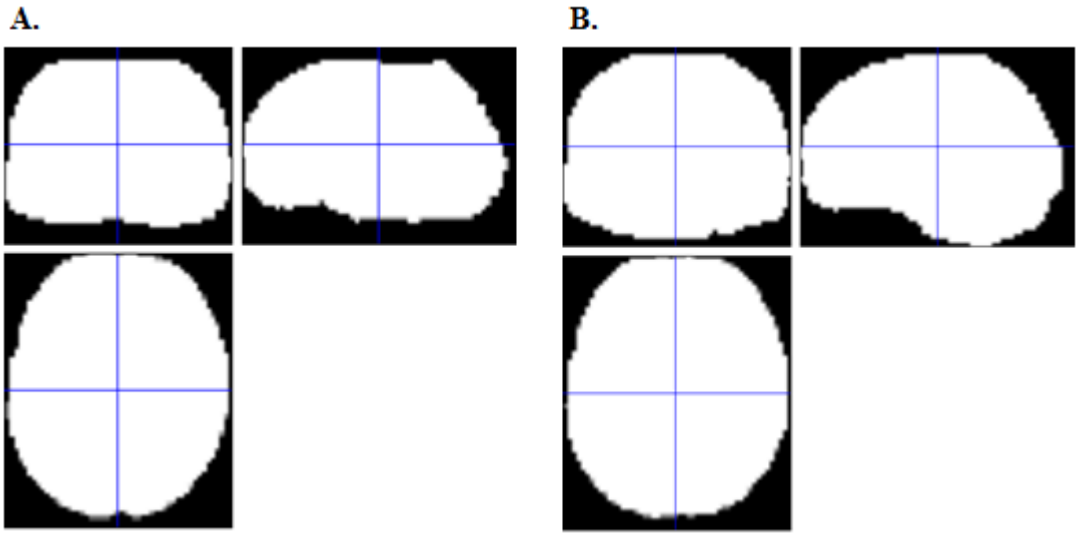
Mask.img for anticipation large win vs. no win (n = 414, voxels in mask: 71383)



Chapter 6:

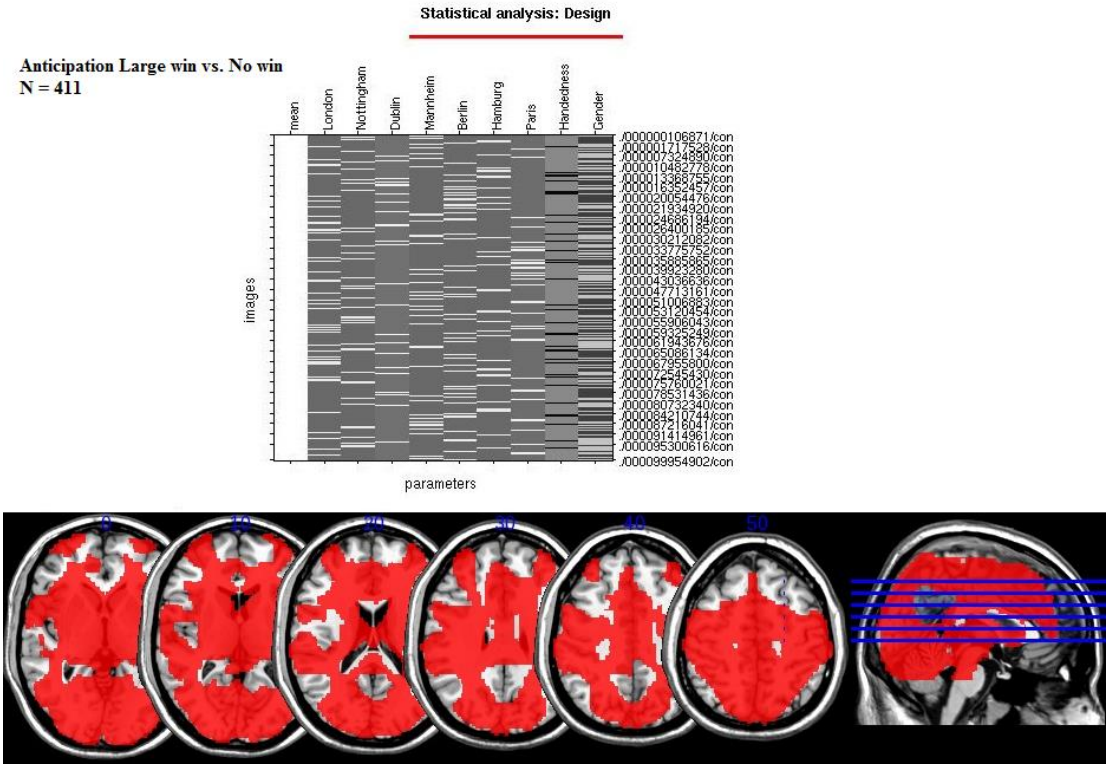
A. Mask.img for anticipation large win vs. no win (n = 190, voxels in mask: 73675).

B. Mask.imag for stop success vs. go success (n = 143, voxels in mask: 78418)

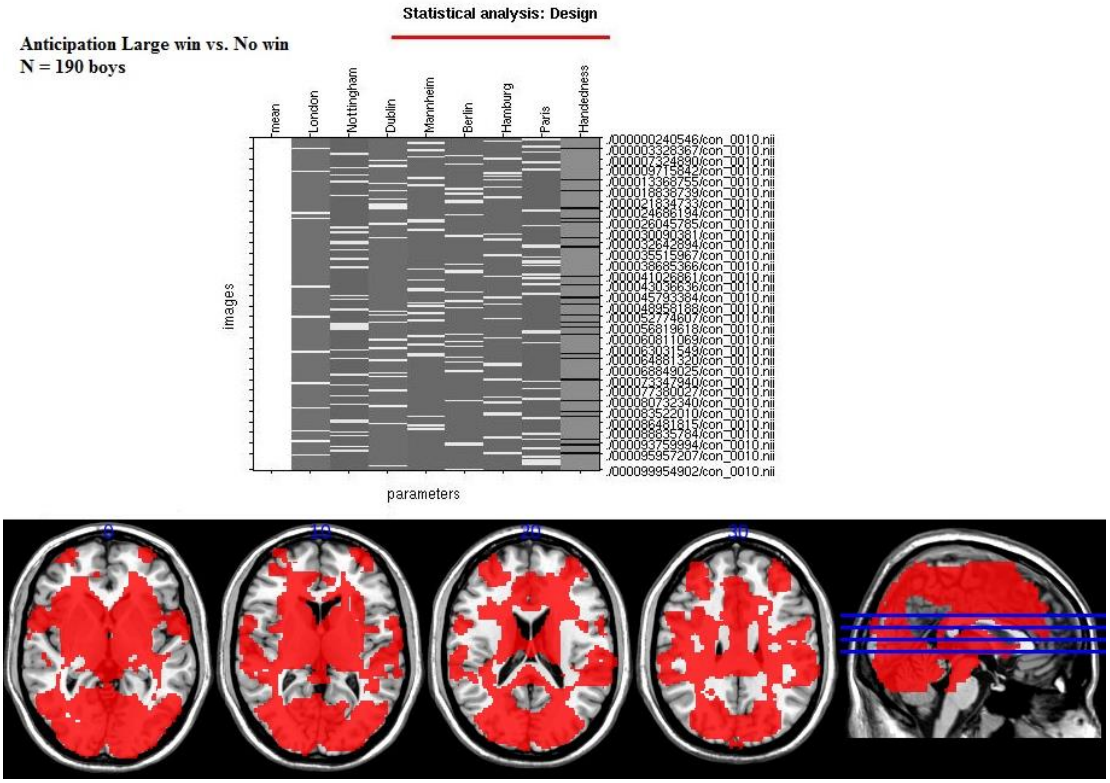


9.8 APPENDIX 6: SECOND-LEVEL MODELS AND RANDOM EFFECTS ANALYSES FOR CHAPTER 5 AND CHAPTER 6

Second-level analysis and activation patterns for data presented in chapter 5: Second level model of anticipation large win vs. no win with 9 regressors (dummy-coded sites, handedness and gender) and the resulting brain activation in the contrast ($p_{FWE\text{-}corrected} < 0.05$, n = 411).



Second-level analysis and activation patterns for data presented in chapter 6:
Second level model of anticipation large win vs. no win with 8 regressors (dummy-coded sites and handedness) and the resulting brain activation in the contrast ($p_{FWE\text{-}corrected} < 0.05$, n = 190).



Second-level analysis and activation patterns for data presented in chapter 6:
Second level model of stop success vs. go success with 8 regressors (dummy-coded sites and handedness) and the resulting brain activation in the contrast ($p_{FWE\text{-}corrected} < 0.05$, n = 143):